Freshwater dinoflagellates as proxies of cultural eutrophication: a case study from Crawford Lake, Ontario

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Abstract

Crawford Lake, Ontario, provides an ideal natural laboratory to study the response of freshwater dinoflagellates to cultural eutrophication. The anoxic bottom waters that result from meromixis in this small (2.4 ha) but deep (24 m) lake preserve varved sediments that host an exceptional fossil record. These annual layers provide dates for human activity (agriculture and land disturbance) around the lake over the last millennium by both Iroquoian village farmers (ca. A.D. 1268-1486) and Canadian farmers beginning ~A.D. 1883. The well established separate intervals of human activity around Crawford Lake, together with an abundance of available data from other fossil groups, allow us to further investigate the potential use of the cyst of freshwater dinoflagellates in studies of eutrophication. Cyst morphotypes observed have been assigned as *Peridinium willei* Huitfeldt-Kaas, *Peridinium wisconsinense* Eddy and *Peridinium volzii* Lemmermann and *Parvodinium inconspicuum* (Lemmermann) Carty. The latter two cyst-theca relationships were determined by culturing and by the exceptional preservation of thecae of *P. inconspicuum* in varves deposited at times of anthropogenic reductions in dissolved oxygen.

Acknowledgements

I have many people to thank for their contribution to allowing me to finish both my undergraduate and graduate degrees.

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List of Abbreviations

˚C	degrees celcius
A	acidification
A(h)	area in hectares
AD	anno domini
Al^{3+}	aluminum ion
AMS	accelerator mass spectrometry
BOD	biochemical oxygen demand
Ca	calcium
cm	centimetre
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DOC	dissolved organic carbon
H_2S	hydrogen sulphide
H_2SO_4	sulfuric acid
HCL	hydrochloric acid
km	kilometre
KOH	potassium hydroxide
KT	Cretaceous- Tertiary
L	litre
LSU	large subunit
Mg	magnesium
m	metre
mm	millimetre
ml	millilitre
NAP	non-arboreal pollen
NIS	non-indigenous species
NO_2	nitrite
NO_3	nitrate
NPP	non-pollen palynonmorph
P	phosphorus
pH	measure of hydrogen ion concentration
rDNA	ribosomal
RNA	ribonucleic acid
sp.	a single species within a genus
spp.	many species within a genus

SSU	small subunit
stdev	standard deviation
TN	total nitrogen
TP	total phosphorus
µg	microgram
µm	micrometer
Z_{max}	maximum depth

About the Chapters

As co-author for Chapter 2 I was responsible for the Crawford Lake dinocyst downcore distribution data as well as the discussion material relevant to my findings at Crawford Lake. This paper has been submitted and accepted to be part of a special proceedings paper for the Dino9 conference in Liverpool, England.

Chapter 3 as first author I was responsible for all the dinocyst data and the majority of the pollen data. I gathered all the background information including archaeological for Crawford Lake as well as all the paleolimnological studies that had previously been done at Crawford Lake. Chapter 3 is to be submitted to the Journal of Paleontology.

Chapter 1: Introduction

1.1 Microfossils as proxies of cultural eutrophication

Cultural eutrophication, the enrichment of aquatic environments with excess nutrients due to human influences, is the most common water quality problem around the world. Phosphorous is the limiting nutrient needed for primary production in temperate lakes (Gloterman et al., 1975; Smith and Schindler, 2009), so controlling phosphates is an important step in controlling eutrophication. Human activity has had detrimental affects on numerous ecosystems and disturbed many natural chemical cycles. Clear cutting of forests increases soil erosion and the release of nutrients into groundwater and surface runoff, and agricultural practices further enhance soil erosion, and fertilizers and animal waste are additional sources of nutrients. Residential communities further enhance nutrient loading from lawn and garden fertilizers and pesticides, and human and industrial waste plays a major role in the overloading of limiting nutrients into waterways from storm sewers and seepage from sewer systems and degrading sewage processing plants. Industries and vehicles play a part in the contribution of excess nutrients from waste and emissions (Ekdahl et al., 2004; Klug and Cottingham, 2001). The input of limiting nutrients, especially during times when nutrient levels are usually low (i.e. late summer/early fall), results in excessive algal and plant growth that leads to a depletion of dissolved oxygen levels (DO) due to increased biochemical oxygen demand (BOD) as decay occurs, as well as a decrease in light penetration. This degradation in water quality in turn leads to the death of both plant and animal life in the aquatic ecosystem (Smol, 2008).

Crashes in fisheries around the world caused by algal blooms (“red tides”) and the decline in the quality of fresh water reservoirs has led to the study of the impact of cultural eutrophication on ecosystems. Long-term trends have to be studied in order to understand the present, and to prevent future degradation of our waterways by implementing sustainable management measures. The most commonly used methods for investigating past eutrophication is the analysis of microfossil records. Measuring the concentration of major nutrients (e.g. phosphorous or nitrites) and biomass and the products of photosynthesis in the water column (e.g. chlorophyll *a* in lake water) provides insights into ecosystem health (Carlson, 1977; Torbick et al., 2008), but these only record a brief snapshot at any particular time (Bradshaw et al., 2002). These synoptic measurements may not accurately reflect conditions in the area. Microfossils, in

contrast, integrate environmental conditions over a period of time and record long-term changes that occur in freshwater systems (Yoder and Rankin, 1998).

In order to sustainably manage and remediate ecosystems for the future, the past must be investigated to identify the cause and the critical overloading point (Dale, 2009). Many proxies (magnetic susceptibility, trace metal concentrations, dissolved oxygen, nitrogen and phosphorus concentrations), have been used to study the variety of aspects associated with cultural eutrophication (Smol, 2008). Time series data made available by microfossil records in sediment cores demonstrate the long-term pattern of cultural eutrophication, particularly when integrated with geochemical analysis of the sediment (Dale, 2009). Using several different records together to form a multi-proxy analysis develops a more reliable diagnostic tool than the use of any given single proxy, since each has limitations. Dinocysts have been employed as proxies of eutrophication in marine environments (Daleet al, 1999; Dale, 2001 & 2009; Harland et al., 2004; Sangiori and Donders, 2004; Brenner, 2005), but the most commonly used proxies in lacustrine environments are diatoms and thecamoebians (testate amoebae).

Diatoms, being the most important primary producers (responsible for ~20% of global photosynthetic fixation of carbon- more than the entire world's rainforests according to Mann (1999), show dramatic changes in assemblages and concentrations with the introduction of excess nutrients that are usually limited (Hall and Smol, 1996; Matsuoka, 1999). Diatoms are photosynthetic algae that have a fossilizable siliceous skeleton (frustule) and are found in almost every aquatic environment including fresh and marine waters (Hall and Smol, 1996). Previous studies (Ekdahl et al., 2004 & 2007; Hall and Smol, 1996) have shown changes to occur in their assemblages and abundance due to human-induced eutrophication. Ekdahl et al. (2004 & 2007) analysed the diatom assemblages and abundance of a core from Crawford Lake and found that the diatom assemblage underwent a complete transformation with the establishment of Iroquoian settlements. The pre-Iroquoian planktonic diatom assemblage in Crawford Lake consisted of meso-oligotrophic *Cyclotella michiganiana* and *Cyclotella bodanica* that was replaced initially by an increase in *Stephanodiscus* due to the initial pulse of human sewage into the watershed. After this initial input of nutrients *Cyclotella distinguenda, Cyclotella glomerata*, and *Cyclotella stelligeroides* appeared rapidly and comprised a significant proportion of the diatom flora (Ekdahl et al., 2007). The most eutrophic conditions are signified by an increase in *Synedra nana, Fragilaria crotonensis* and *Asterionella formosa,* indicating increased concentrations of phosphorous (Ekdahl et

al., 2004). Benthic diatom assemblages also changed following Iroquoian disturbance: *Nitzschia* species, *Achnanthidium minutiisimum* and *Amphora libyca* increased and *Cymbella delicatula, Navicula radiosa*, and *Staurosira pinnata* appeared (Ekdahl et al., 2007). Once the Iroquoian people left the area and nutrient levels returned to pre-Iroquoian values, diatom abundances returned to those recorded during the pre-Iroquoian zone, but diatom assemblages remained in their altered state. When Euro-Canadian settlers started to farm the land around Crawford Lake the diatom assemblages did not change further, but their abundance far exceeded that seen during the Iroquoian zone. It was concluded that the initial introduction of anthropogenic nutrients in the 13th century permanently altered the Crawford Lake phytoplankton communities. Increased primary productivity (diatoms, dinoflagellates, and other phytoplankton) led to increased oxygen use through respiration and degradation of organic matter (biochemical oxygen demand- BOD), significantly decreasing the dissolved oxygen content (DO) of the basin's bottom waters (Ekdahl et al., 2004). The resulting anoxia produced the well preserved, undisturbed laminated sediments (varves).

Thecamoebians (testate amoebae) have also proven useful in documenting cultural eutrophication, including several studies in Southern Ontario lakes (Reinhardt et al., 2005; McCarthy et al., 2011, Danesh et al., accepted). One thecamoebian species in particular, *Cucurbitella tricuspis*, "blooms" in response to high nutrient availability, and because this thecamoebian taxon has a planktonic phase, it can avoid the very low DO at critical times (primarily in the late summer). The increased nutrient flux to the Frenchman's Bay watershed (~40 km east of Toronto) led to an increase in *Cucurbitella tricuspis* from 10-20% to 40-70%, correlating with a marked increase in magnetic susceptibility (Reinhardt et al., 2005). Prior to human activities around the bay, the thecamoebian assemblages were diverse, consisting of *Centropyxis* spp., and *Difflugia* spp (Reinhardt et al., 2005). Thecamoebians from Sluice Pond, Massachusetts record eutrophication beginning ~4000 years ago, initially due to evaporative conditions reducing water levels in the lake and subsequent warming, and later related to human activities over the last few centuries (Hubeny et al., in prep.). A dramatic increase in thecamoebian concentrations and diversity resulted from the natural eutrophication, but numbers of thecamoebians and species diversity declined with cultural eutrophication due to a decrease in dissolved oxygen in the bottom waters and the onset of meromictic conditions, with only the most tolerant species (*Cucurbitella tricuspis* and *Difflugia protaeformis*) thriving on the modern lakebed. These species thrived with recent human

impact in Cook's Bay (Lake Simcoe, Ontario) as well, together with a ciliate protozoan, *Codonella cratera* (Danesh et al., accepted).

1.2 Palynomorphs as proxies of cultural eutrophication

Palynomorphs are sedimentary particles between 5 and 500 µm in size made of acid-resistant complex organic material such as chitin, pseudochitin (tectin), sporopollenin, or dinosporin (Benton and Harper, 1997). Palynology studies these particles, which are produced by organisms from every kingdom of life (Monera, Fungi, Protista, Plantae, and Animalia) and are common in sediments and sedimentary rocks of Precambrian to Holocene. In lake sediments, the most abundant palynomorphs are usually pollen grains together with the acid-resistant remains of various protistan algae (e.g. desmids and dinoflagellates) (Benton and Harper, 1997; Traverse, 1988).

Pollen, the male gametophytes of seed-bearing plants, as well as the spores of embryophyte plants (both made of sporopollenin), have been the most commonly studied palynomorphs since the pioneering work of Lennart von Post in the early 20th century. While pollen and spores do not reflect limnological conditions, they do provide insights into disturbance of the vegetation in the watershed, and thus are useful in studies of cultural eutrophication. A sharp increase in non-arboreal pollen such as ragweed (*Ambrosia sp.*) usually indicates the clear cutting of forests for human use, housing and agriculture. There are factors other than human impact that affect plant distribution (mainly climate). However, natural forest fires, for instance, can mimic human clear cutting.

Pollen records have been extremely useful at identifying the occurrence and timing of human impact in the area of Crawford Lake. The pollen of cultivars such as *Zea mays* (corn), along with *Cucurbita* (squash), *Helianthus* (sunflowers), *Portulaca* (purslane) (Boyko, 1973; Byrne and McAndrews, 1975; McAndrews & Boyko-Diakonow, 1989; Byrne, 1998) as well as spores of fungal parasites on cultivars, such as *Ustilago maydis* (corn smut) (McAndrews and Turton, 2010) identifies Iroquoian agriculture between the 13th and 15th centuries. Abundant ragweed (*Ambrosia*) and other non-arboreal (herbaceous) pollen, records widespread deforestation by Euro-Canadian settlers dated ~1867 A.D. by varve counting (Boyko, 1973; McAndrews & Boyko-Diakonow, 1989). Re-forestation is evident from the resurgence of arboreal (tree) pollen in the intervening centuries.

Non-pollen palynomorphs are common in most lacustrine sediments, and they can provide insights into human impact and eutrophication (Haas, 2010). A variety of non-pollen palynomorphs were employed in studies of cultural eutrophication in lakes from southern Ontario (e.g. Burden et al., 1986; Turton and McAndrews, 2006; McCarthy et al., 2011; Danesh, 2011). A multiproxy approach, involving chemical analysis of water quality indicators as well as various microfossil groups was used in a study of Lake Simcoe water quality (Danesh et al., accepted). A variety of non-pollen palynomorphs, such as desmids, dinoflagellates, and chlorophyte algae (e.g. *Pediastrum*) and various types of protozoa were shown to respond to various episodes of anthropogenic impact, beginning in the late 18th Century (Fig. 1.1 a & b).

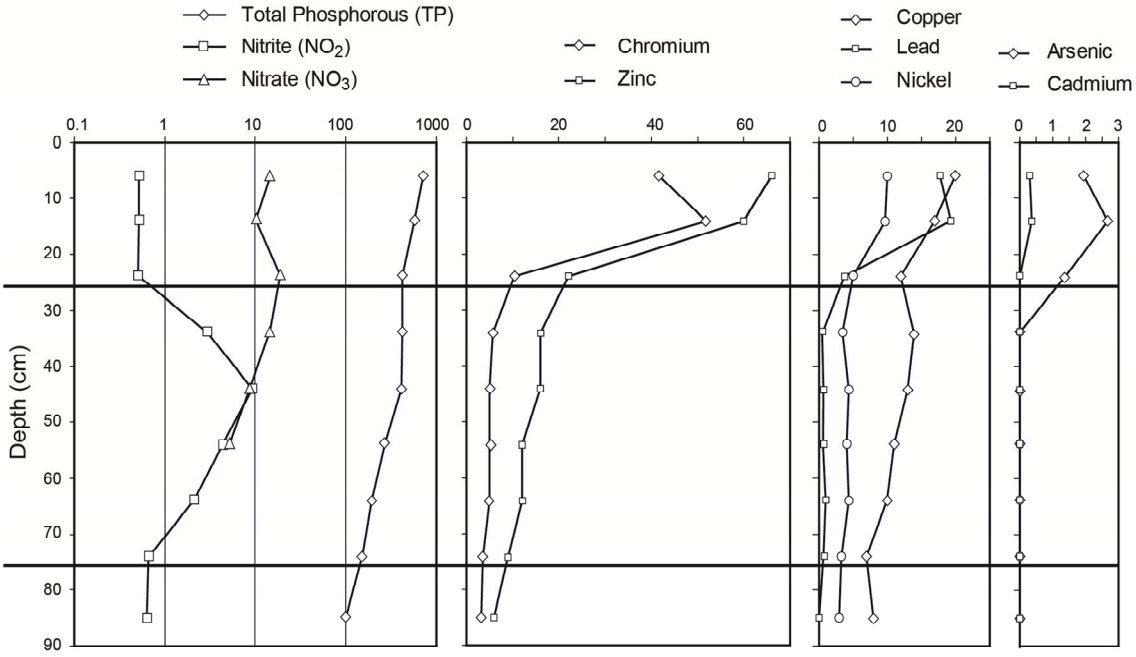


Fig. 1.1 a Total phosphorus (TP) concentrations increase rapidly and more-or-less steadily in this core from Cook's Bay, Lake Simcoe, recording increased anthropogenic impact in the Cook's Bay watershed beginning around 75cm in the core. Nitrite (NO_2) concentrations rise sharply to 9.29 mg/kg at 44 cm, when concentrations of nitrate (NO_3) begin to rise steadily, probably in response to a decline in dissolved oxygen (DO) in bottom waters associated with microbial decomposition of organic influx from the Holland Marsh when polders were created in the 1920's and 30's. The concentrations of heavy metals remain low until 24 cm, when ragweed becomes abundant (Danesh et al., accepted).

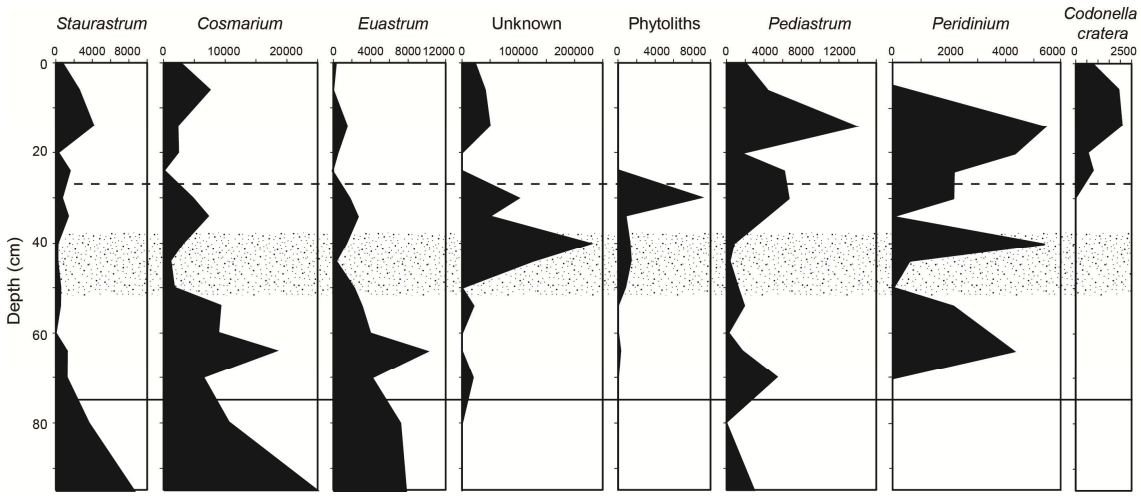


Fig. 1.1 b Major changes in assemblages of non-pollen palynomorphs appear to respond to anthropogenic impact. Desmids, (*Staurastrum* spp., *Cosmarium* spp., *Euastrum* spp.) are most abundant below 75 cm, recording oligotrophic environments prior to the construction of the Toronto Carrying Place and settlement of Aurora and Newmarket in the late 18th C. Abundances of *Pediastrum* and *Peridinium* are relatively low to absent in the lower part of the core but become abundant above 75 cm, indicating anthropogenic disturbance and higher nutrient levels. The presence of phytoliths and an unknown non-pollen palynomorph between 50cm and 40cm of the core (stippled) is probably due to the drainage of the Holland Marsh and resulting organic flux into Cook's Bay during the 1920's and 30's. The presence of *Codonella cratera* in the upper part of the core records low DO, probably resulting from high BOD since the end of the Second World War, when the Cook's Bay watershed became intensely urbanised (Danesh et al., accepted).

Rotifers are microscopic aquatic herbivores that can be found in many freshwater environments. They play an important ecological role in the natural water purification process, since their diet consists of decomposing organic matter and phytoplankton. Their loricas (hard outer sheaths) and eggs are acid-resistant, and have a fairly high preservation potential, particularly in low-oxygen conditions. Small numbers of rotifer loricas were identified in sediments from Lake Simcoe (Danesh, 2011), but Turton and McAndrews (2006) found an abundance of rotifer loricas and eggs in the varved sediments from Crawford Lake. The organic remains of rotifers were particularly abundant during the periods of human influence in and around the lake, reflecting nutrient-induced phytoplankton blooms (Turton and McAndrews, 2006). Turton and McAndrews (2006) reported that the abundance of rotifer loricas (*Keratella* sp. and *Kellicottia* sp.) covaried with the abundance of diatoms, correlating with the two distinct periods of human activities around Crawford Lake reflecting increased phytoplankton production due to an influx of limiting nutrients.

A few studies have used dinoflagellate cysts (dinocysts) in marine environments to examine eutrophication (Dale, 2009, Dale et al., 1999, Matsuoka, 1999) as a result of local fisheries collapsing due to algal blooms that killed off fish stocks, but little work has been done to evaluate the potential of dinocysts as proxies of cultural eutrophication in lacustrine systems (Burden et al., 1986; Zippi et al., 1990 & 1991; Chu et al., 2008). Their distribution in a core from Honey Harbour, Ontario (Fig. 1.2), however clearly illustrates their potential in studies of cultural eutrophication (McCarthy et al., 2011).

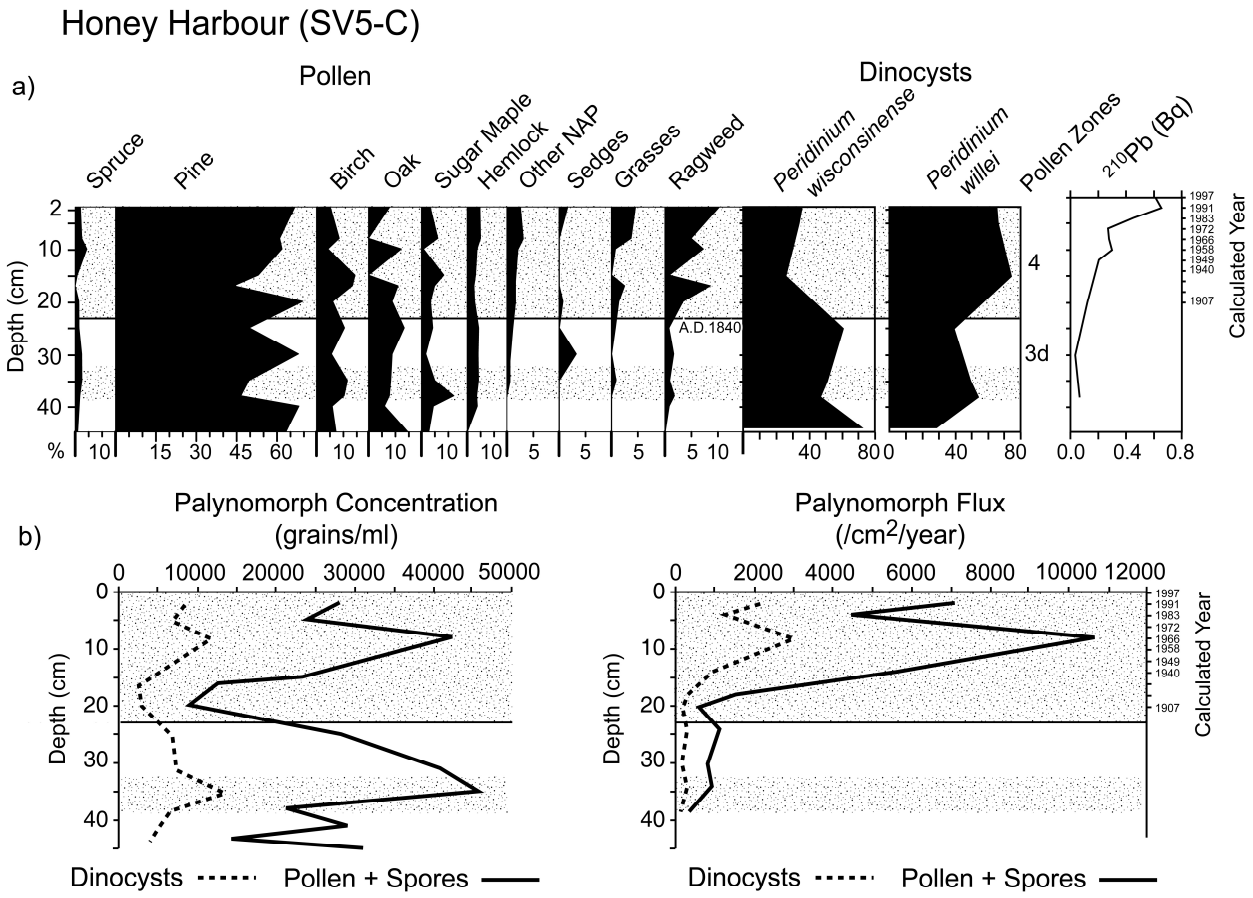


Fig. 1.2 Increases in dinocyst abundance and flux and a relative increase in *Peridinium willei* correspond to human impact from Wendat and subsequent Euro-Canadian settlement (stippled) in a core from Honey Harbour, Ontario (from McCarthy et al., 2011).

1.3 Freshwater dinoflagellates and their cysts

Dinoflagellates are microscopic, unicellular, flagellated protists that are a major constituent of the plankton assemblage of most aquatic environments. They are an important component of the summer phytoplankton of North American lacustrine

ecosystems (Carty, 2002; Kohler and Clausing, 2000), and although the first extant freshwater dinoflagellate was described by Schilling (1891), freshwater dinoflagellates have been widely overlooked, especially relative to their marine counterparts. They are very poorly described compared to other phytoplankton organisms, and their cytology, physiology, biochemistry, life cycles, reproduction, and ecology are very poorly known, although key factors controlling their distribution are thought to be temperature, nutrient availability, and pH (Popovsky and Pfiester, 1990) (Figure 1.3). Their biogeographic distribution is relatively unknown compared to their marine counterparts due to lack of knowledge, study aim, and misinterpretation of cysts, thus information of their organic cysts in sediments and their distribution is very scarce.

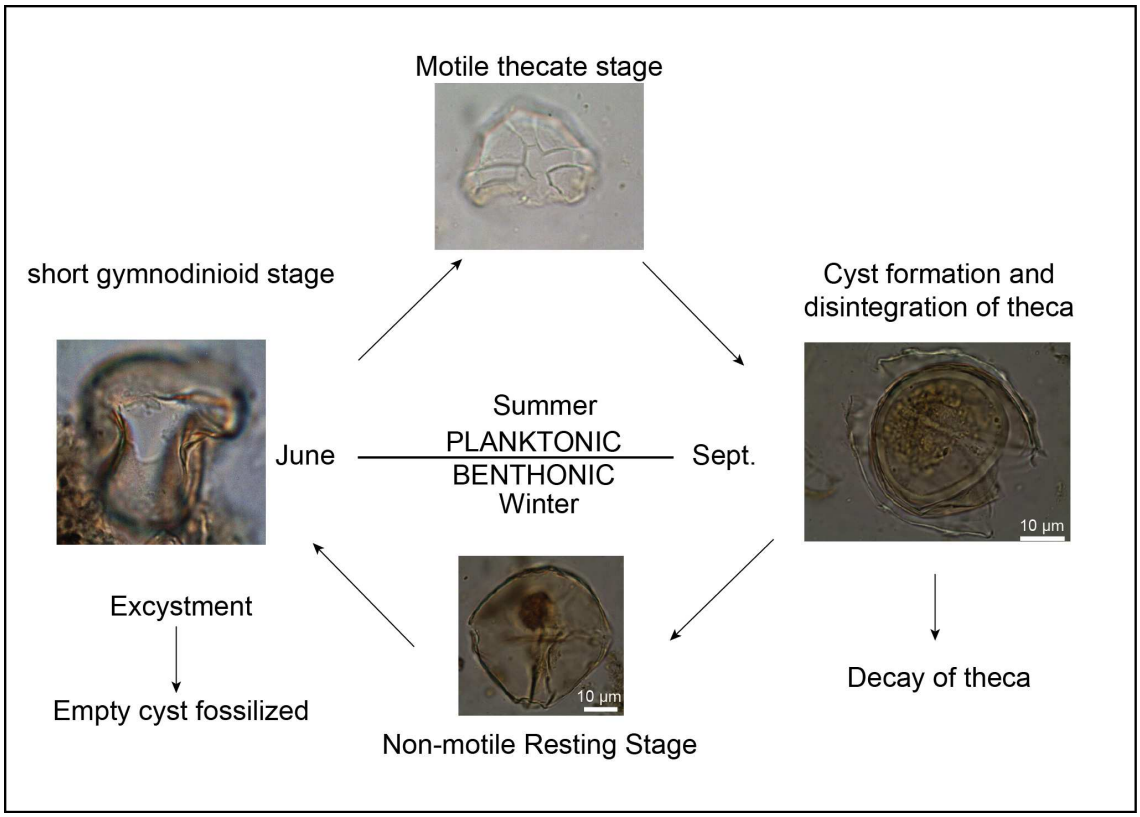


Figure 1.3 Life cycle of *Peridinium volzii* Lemmermann modified from Wall and Dale (1968).

Few studies have focused on freshwater dinocysts and their use as paleolimnological tools: Europe (Bourrelly and Coute, 1980; Hickel and Pollingher, 1986; Evitt et al., 1985; Kouli et al., 2001, Tardio et al., 2006 a & b; Kohler and Clausing, 2000) Asia (Li et al., 1992; Wu and Chou, 1998; Wang et al., 2004; Chu et al., 2008) North America (Batten et al., 1999; Burden et al., 1986; Evitt and Wall, 1968; Norris and

McAndrews, 1970; Zippi et al., 1991 & 1990; Kim et al., 2004; Wall and Dale, 1968;McCarthy et al., 2011) and South America (Hargraves and Viquez, 1981). Further, comprehensive research on dinocysts in lake sediments as well as in laboratory encystment/excystment experiments and DNA analysis is needed for their successful use in environmental interpretations.

The most common genus in freshwater environments is *Peridinium,* with thecae ranging in size from ~10 - 100 µm in length and made of approximately 20 plates of a cellulose-like complex polysaccharide. The arrangement ("plate tabulation": 4', 2-3a, 5-6c, 7'', 5''',2'''') and ornamentation of thecal plates is used to identify species (Figure 1.4), but this is not straightforward, due to infraspecific variation. Carty (2008) separated the Umbonatum group (*P. umbonatum, P. inconspicuum, P. centenniale*) into a new genus *Parvodinium* based on their small size (12-20 µm), presence of an apical pore and 2 intercalary plates. A wide range of morphotypes can occur within species (Popovsky and Pfiester, 1990). Kim et al. (2004) found two morphologically similar, but genetically distinct, populations of the microbial eukaryote *Peridinium limbatum* (Stokes) Lemmermannn from Crystal Lake and Crystal Bog (Oneida Co., Wisconsin). The greater genetic variation between the two *P. limbatum* populations than that reported in the literature for some morphologically distinguishable microalgal species suggests the occurrence of cryptic sister species, but hybrid sequences obtained from one of the Crystal Lake strains suggest that the two populations may still be members of a single sexually compatible biological species (Kim et al., 2004).

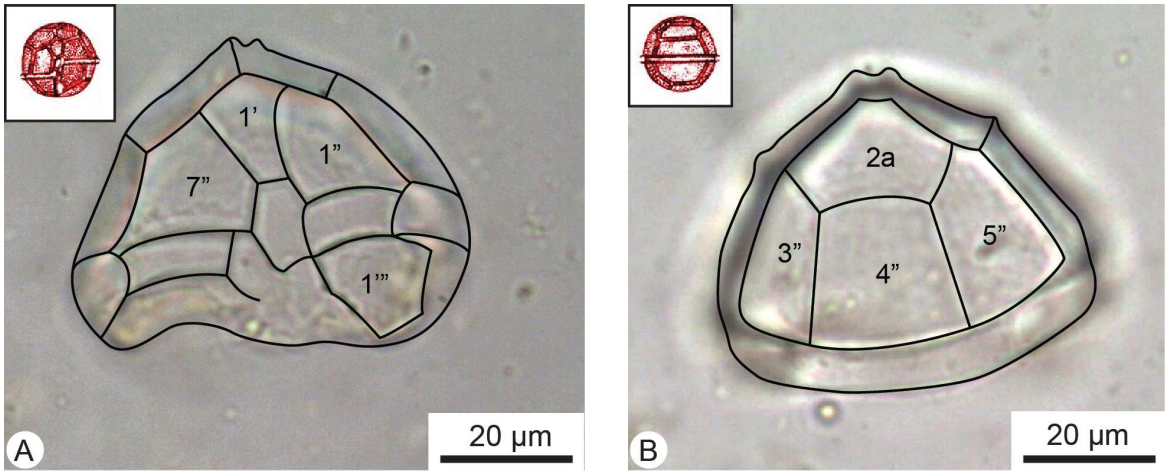


Figure 1.4 A & B *Peridinium volzii* Lemmerman thecae exceptionally preserved in palynological preparations from varved sediments in Crawford Lake illustrating the tabulation in the genus *Peridinium* (4', 2-3a, 7'', 5-6c, 5''',2''''). (Insets of schematic drawings modified from Shen et al., 1990.)

Dinoflagellates bloom in response to nutrient flux, and although unlike their marine counterparts, freshwater taxa do not have toxins (Carty, 2002; Popovsky, 1983). Blooms of freshwater dinoflagellates can have detrimental effects by: 1) restricting sunlight, 2) depleting oxygen levels (BOD), and 3) causing a taste and odour problem with drinking water, making it unfit for human consumption. Wall and Dale (1968) found that cysts form in association with dinoflagellate blooms. In addition to the relatively short-lived hypnozygotes that participate in sexual reproduction, some species of dinoflagellate produce longer-lived resting cysts of dinosporin that preserve in the fossil record. Dinoflagellate blooms (freshwater “red tides”) are recorded in laminated sediments from Lake Xiaolongwan, NE China that consist of a dinoflagellate cyst-rich layer coupled with a grey organic and siliceous rich layer (Chu et al. 2008). They used sediment traps to determine whether internal (seed banks, germination and endogenous clock) or external (temperature, light, nutrients, species composition, currents, anoxia) factors (or both) play a role in dinoflagellate bloom dynamics (Horne et al., 1971), and determined that dinoflagellate blooms coincide with autumn turnover when nutrient levels are high and cysts are brought up from the lake bottom and germinate.

Wall and Dale (1968) studied the theca-cyst cycle of modern marine and fresh-water dinoflagellates in order to establish detailed correlations between living and fossil dinoflagellates. Selected dinoflagellate cysts were incubated in order to aid in comparison and correlation with the variable systematic nomenclature, resulting from the study of cysts by palynologists and the study of thecae by phycologists using different nomenclature for the different stages of a single species. Samples containing *Peridinium limbatum* and *Peridinium wisconsinense* were collected from Round Pond, Falmouth Massachusetts and incubated in the laboratory. Capsulate cysts that formed were identical to those found (but misidentified as thecae) by Eisenack and Fries (1965) from Weber Lake, Minnesota and named *Peridinium limbatum minnesotense*, and *Peridinium wisconsinense*. Both species posses capsules which they have in common with older marine *Protoperidinium* species, suggesting an invasion of freshwater environments during the Palaeogene or early Neogene when marine species still produced a capsulate cyst. *Peridinium limbatum* and *P. wisconsinense* have a distinctive archeopyle structure compared to their marine counterparts. Although freshwater species retain a pentagonal (peridinioid) shape, including one apical and two antapical horns or lobes, and the 7”, 5” Op and 2”” pattern of tabulation formulae for peridinacean dinoflagellates.

Alster et al. (2006) attempted to determine the cause and time of encystment of *Peridinium gatunense* in regards to nutrients, light intensity, temperature, and time of year for tropical freshwater Lake Kinneret northern Israel. *P. gatunense* has a seasonal bloom from winter-spring and collapses during summer stratification and lake temperatures over 30°C. They found a positive correlation between vegetative cell density in the water column and cyst abundance in the sediments. In laboratory encystment experiments only samples collected from April to May were successful coinciding with their motile counterparts exponential growth phase, all other samples collected from other times during the year were unsuccessful thus encystment must occur during the same time period in Lake Kinneret itself. The bloom period also correlated with the time of year when nitrate concentrations are at their peak. Percentage of encystment is the greatest (~3%) just before the peak of the bloom compared to the average of 0.1% during the whole duration of the bloom and also coincides with the highest growth rate of motile cells. There was no correlation between light intensity, temperature and phosphorus concentrations on encystment observed during the laboratory encystment experiments. Dinoflagellates may be able to detect minor fluctuations in nitrate concentrations as their greatest rate of encystment coincides with the onset of decreasing nitrate concentrations in the spring.

1.4 Non-marine dinoflagellates in the fossil record

Dinoflagellates have a widespread distribution and a long marine fossil record. Traverse (1955) published the first fossil finding of freshwater dinocysts from the Oligocene (*Peridinium hansonianum*), a taxon revisited by Evitt (1974) who followed (Norris and Hedlund, 1972) in suggesting that certain Mesozoic dinoflagellate taxa found refuge in freshwater environments during the early Cenozoic. Norris and McAndrews (1970) tentatively attributed cysts (types A-D) in Holocene sediments from Minnesota to four extant species, based on gross morphology: *Peridinium limbatum*, *Peridinium wisconsinense*, *Peridinium willei*, and *Peridinium bipes*. Norris and McAndrews (1970) cited a culturing study published by Evitt and Wall (1968) as well as modern biogeographic distribution to attribute cyst type A to *P. limbatum*, and they more tentatively attributed cyst type B to *P. wisconsinense* on similar grounds. The biogeographic distribution of *P. bipes* made it unlikely that cysts of this species were represented in the sediments from Glatsch Lake, however, and subsequent work by McCarthy et al. (2011) suggests that both types C and D can be attributed to *P. willei*.

Batten et al. (1999) and Kohler and Clausing (2000) attributed monospecific assemblages of cysts found together with other lacustrine phytoplankton in Cenozoic sediments to freshwater dinoflagellates. Batten et al. (1999) identified cysts comparable to that of *Pseudokomewia granulata* (He, 1980), known only from Oligocene successions in China, in non-glacial varved lacustrine deposits of the Miocene Clarkia lake succession in Northern Idaho. The anoxic environment implied by varved sediments favoured the preservation of dinocysts together with other palynomorphs and organic matter, whereas taphonomic factors are probably responsible for the absence of palynomorphs in lacustrine deposits over- and underlying the varves. Kohler and Clausing (2000) found well-preserved cysts associated with siderite layers suggesting a Ca-rich environment in sediments covered by a basaltic blanket from the upper Oligocene fossillagerstatte Enspel in the Westerwald area of Germany. This, together with the monospecific nature of the assemblage, comprised of a new species of freshwater dinoflagellate cyst, *Cleistosphaeridium lacustre*, indicates specific environmental condition, most likely related to the pyroclastic setting. Dinoflagellates represent a major part of the primary producers relative to other phytoplankton (diatoms, chrysophytes, green algae and cyanobacteria) found in the sediments, and the phytoplankton composition of the sediments from Lake Enspel suggests seasonal successions with both climate and ecology controlling the associations.

Most sources state that resting cysts of dinosporin are the only fossilizable dinoflagellate remains, although exceptional preservation of thecae (the vegetative stage) and hypnozygotes has been noted in lagerstatten from Crawford Lake (Krueger et al., 2011; McCarthy and Krueger, 2012; Turton, personal commun.) and in amber by Masure and Vrielynck (2011). The preservation of both thecae and cysts of several species of *Peridinium* in varved sediments from Crawford Lake (Krueger et al., 2011) provides further insights into the affinity of cysts (Chapter 3) as well as their ecological tolerances (Chapter 4).

1.5 Dinocysts as paleolimnological proxies: potential and limitations

The presence of freshwater dinocysts is often cited in broader paleoecological studies in order to demonstrate a freshwater depositional environment or freshwater flux to neritic environments, but these studies typically have little or no taxonomic detail (e.g. Miller et al., 1982; Findlay et al., 1998; Matthiessen et al., 2000). While it is fairly easy to identify the dinoflagellate affinity of a cyst, identifying it to species level is not straightforward.

Theca-cyst relationships remain poorly understood, and the affinity of cysts to species has usually been inferred on the basis of gross morphological similarities and biogeographic distribution. (e.g. Evitt and Wall, 1968; Norris and McAndrews, 1970; Burden et al., 1986), but cysts of *Peridinium willei* Huitfeldt-Kaas and *Peridinium wisconsinense* Eddy have been confirmed through germinations of both cyst species as well as single-cell LSU rDNA analysis on an excysted cell of *Peridinium willei* (McCarthy et al., 2011).

Even in studies where both phycological and palynological approaches are combined, it has proven difficult to definitively relate thecae in the water column with cysts preserved in the sediment. Tardio et al. (2006 a & b) discovered peridinioid dinoflagellate cysts in Lake Nero de Cornisello, Italy, where two species of dinoflagellates, *Peridinium cinctum* and *Peridinium umbonatum* were identified in the water column. Three cyst morphotypes, all ~ 20 X 25-30 µm in size, found in all the downcore sediment samples analyzed: 1) elongated cyst with a pointed to round antapical end that displays a trapezoidal archeopyle (the most common morphotype), 2) elongated cyst with a pointed to round antapical end with striations and a hexagonal archeopyle, and 3) rounded cyst with a trapezoidal archeopyle. Further culturing and DNA sequencing are needed to determine cyst-theca relationships and evaluate whether some are ecophenotypes.

Chu et al. (2008) attempted to relate the cysts to one of the two living species of dinoflagellates found in the water column of Lake Xiaolongwan: *Peridinium volzii* Lemmermannn (the most abundant) and *Ceratium furcoides* (Levander) Langhans with the cysts making up laminations but concluded (on morphological grounds) that the cysts in the sediment do not correlate with the dinoflagellates found in the water column. They attributed the cysts to *Parvodinium inconspicuum* (Lemmermann) Carty, but recent work on laminated sediments from Crawford Lake (Krueger et al., 2011; Chapter 3) suggests that the cysts that form the organic laminae in Lake Xiaolongwan are not *P. inconspicuum.* The abundant thecae of *Parvodinium inconspicuum* (~ 18-24 µm in diameter, with a prominent cingulum and sulcal ridge, a tiny apical horn and scattered antapical spines) are found together with smooth-walled dinosporin resting cysts in palynological preparations from varved sediments in Crawford Lake. These tiny (~16-20 µm in diameter), unornamentated cysts with a barely visible sulcul indentation and red bodies indicating viable cell contents are attributed to *P. inconspicuum* (see Chapter 3). The observation in the same slide of a nearly identical spongy-walled hypnozygote

undergoing meiosis, identical to hypnozygotes of *P. inconspicuum* illustrated by Pfiester et al. (1984), confirms the affinity of this tiny, unornamented resting cyst. Cysts attributed to *Peridinium volzii*, in contrast (see Chapter 3), are considerably larger (~35-45 µm in diameter) and lack any ornamentation or apical flange but forms two shoulders in the sulcal region (Carty, 2003). These were associated with preserved thecae (including one specimen photographed encysting within a theca (see Chapter 3), allowing cysts initially identified as "small *Peridinium willei*" to be definitively attributed to *Peridinium volzii*. These do not appear to be the cysts illustrated by Chu et al. (2008), despite the abundance of *Peridinium volzii* in the water column of Lake Xiaolongwan.

It is not known how many species of freshwater dinoflagellate produce fossilizable cysts, although dinosporin resting cysts have been associated with various *Peridinium* spp. Most reports of cysts of *Peridinium* in core studies are from acidic eutrophic lakes (e.g. Burden et al., 1986; Zippi et al., 1990 & 1991; Kohler and Clausing, 2000; Tardio et al., 2006 a & b; Chu et al., 2008) although they were also fairly abundant in marly sediments deposited in Lake Simcoe over the last few centuries (Danesh, 2011; Danesh et al., accepted). It is also unclear whether the lack of reports of dinocysts from lakes with abundant dinoflagellates in the water column reflects a true absence, or merely that they have been overlooked by palynologists. Processing techniques can also affect the presence of palynomorphs on slides, as dinocysts are susceptible to oxidization and protoperidiniacean cysts are the least resilient (Hopkins and McCarthy, 2002). Dinocysts may be destroyed or degraded in palynological samples processed using KOH (potassium hydroxide), acetolysis and other oxidizing agents. Thecae are even less resilient than cysts and their presence may also be overlooked.

The influence of taphonomic factors on the biogeographic and stratigraphic distribution of cysts has not been well studied. Even within Severn Sound, McCarthy et al. (2011) found that cyst concentrations on the lakebed varied from <200 to >8500 cysts per gram of sediment. This may reflect variations in DO within Severn Sound, even with subtle variations in water depth, as was measured by McCarthy et al. (2011) in Honey Harbour [both spatially (Table 1.1) and temporally (Fig. 1.5)]. Another possibility is that these light, silt-sized particles are winnowed and re-sedimented by the relatively strong bottom currents in parts of Severn Sound (McCarthy et al., 2007). The extraordinary preservation of cysts in H_2S-rich, black gelatinous gyttja in Honey Harbour, a restricted arm of Severn Sound,is probably due in part, to the low DO (<0.25 mg/L) measured in parts of Honey Harbour, particularly just before fall turnover (McCarthy et al., 2011).

More work must be done to determine dinocyst distribution in sediments and their relationship to ecological parameters as well as taphonomic factors since oxidation and re-sedimentation appear to be important factors.

Table 1.1 Measurements of dissolved oxygen (DO), water temperature and pH from nearshore environments off docks at 44.86^{o}N, 79.82^{o}W in Honey Harbour were made using a Hydrolab on October 13, 2009 (McCarthy et al., 2011).

Site	Aquatic vegetation	Sediment Description	z(m)	DO (mg/L)	pH	Water temp.(^{o}C)
#1	Minor	surface brown muds above gelatinous black gyttja, strong H_2S odor	1.2	0.25	7.3	9.6
#2	Abundant	Loose brown muds, No H_2S odor	1.0	8.7	7.3	10.1
#3	Absent	dark brown highly gelatinous gyttja, strong H_2S odor	2.2	0.05	7.3	9.6

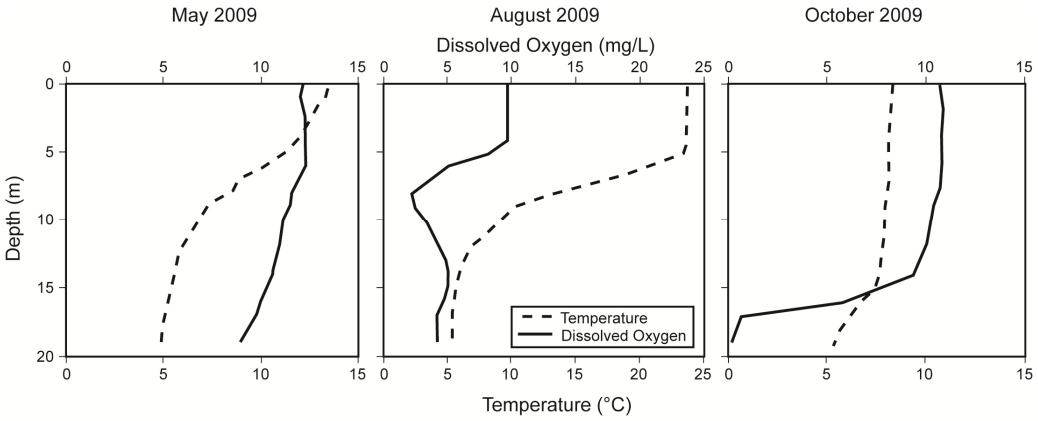


Fig. 1.5 Measurements of temperature and dissolved oxygen (DO) concentrations through the water column in Honey Harbour. Note the sharp seasonal thermocline that develops through the spring and summer, and the nearly anoxic conditions above the lakebed in the autumn (McCarthy et al., 2011).

Although there is still much important work needed to determine theca-cyst relationships and cyst taxonomy, as well as studying the ecological and taphonomic controls on their distribution on lakebeds, there is much that we can infer by comparing the downcore distribution of cysts of *Peridinium* with that of other better-understood proxies (e.g. diatoms, *Pediastrum*, other non-pollen palynomorphs), or in historic

sediments where the degree and types of disturbances are well documented (e.g. pre- and post- *Ambrosia* rise in eastern North America).

Burden et al. (1986), for instance, related downcore variations in *Peridinium* cysts in two small lakes on the southern Georgian Bay coast to changes in land-use over the last six centuries accompanying two distinct phases of human settlement: 1) ~A.D. 1450-1650 by First Nations people (Wendat/ “Huron”) who farmed the land, and 2) when European settlers cleared land to establish communities in the Midland region, recorded in ragweed-rich sediments deposited since ~A.D. 1840 in nearby Wye Marsh (Chittenden, 1990). Cysts which they attributed to *Peridinium willei* and *Peridinium wisconsinense* were identified in Second Lake (Fig. 1.6), but only cysts attributed to *P. willei* from nearby Lake Gignac, perhaps because Lake Gignac is less acidic, inhibiting *P. wisconsinense*. They found a positive relationship between *P. willei* and *Pediastrum* in both lakes, suggesting that *P. willei* responds to an increase in nutrients and siltation accompanying both phases of human settlement. *Peridinium wisconsinense* showed an inverse relationship with *Pediastrum* spp., being most abundant in the two centuries between human settlement, when the area was quickly reforested (Burden et al., 1986) and in the upper 10 cm, probably suggesting rapid improved water quality since the establishment of the provincial park in the mid 20th century.

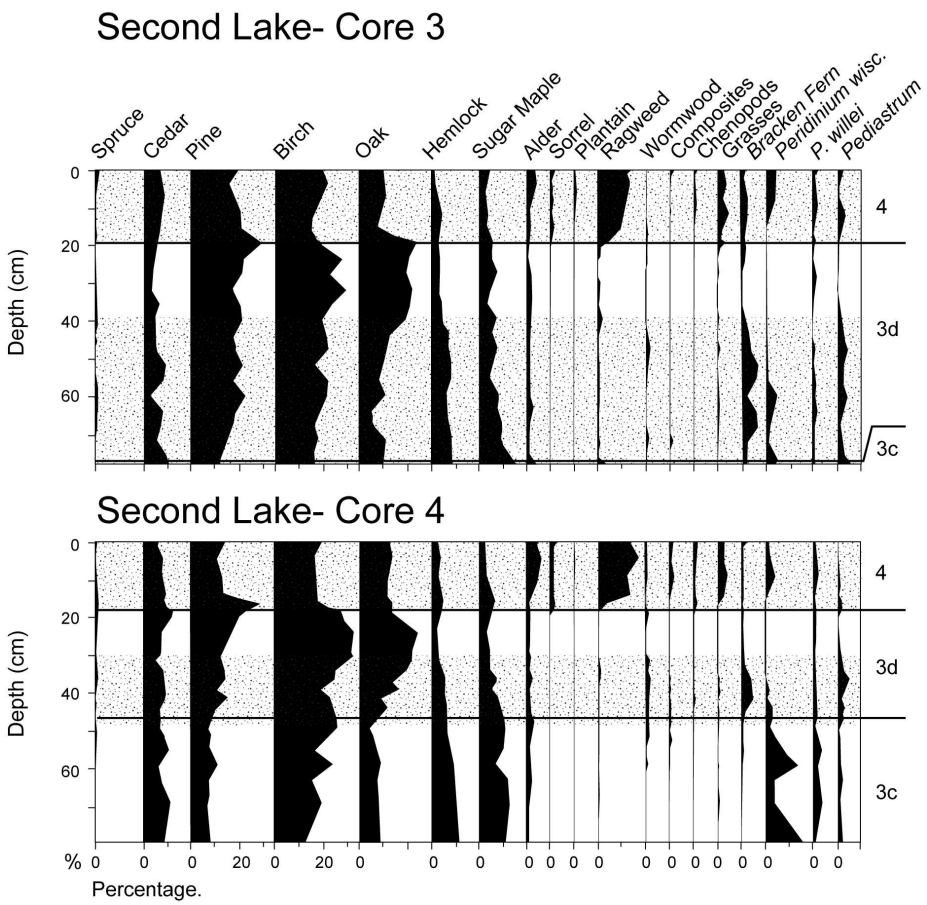


Fig. 1.6 Phytoplankton (*Peridinium wisconsinense, Peridinium willei* and *Pediastrum*) responded positively to the two documented intervals of settlement in the Penetang Peninsula, stippled (Wendat/ "Huron" ~A.D. 1450–1650, and Euro-Canadian, since A.D. 1840), but the relative abundance of *Peridinium wisconsinense* appears to decline with peak impact, illustrated by abundant Pediastrum, a well-documented proxy of eutrophication (modified from Burden et al., 1986).

Zippi et al. (1990 & 1991) studied 11 lakes in the Haliburton-Muskoka region of Ontario. While studying zygnematacean zygospores usefulness as paleoindicators of acidification, Zippi et al. (1990 & 1991) found that *Pediastrum* spp*.* and *Peridinium spp.* may be more useful. Both *Pediastrum* spp*.* and *Peridinium* spp*.* exhibit clear pH preferences, with a higher diversity of *Peridinium* cysts replacing that of *Pediastrum* coenobia with decreasing pH. They found four dinocyst species that they assigned to *Peridinium willei, Peridinium bipes, Perdinium limbatum* and *Peridinium wisconsinense* on gross morphological grounds following Norris and McAndrews (1970). Cysts attributed to *Peridinium willei* and *P. bipes* were found throughout most of the lakes studied and tolerate a wide range of pH values (5.5 – 8.7). On the other hand *P.*

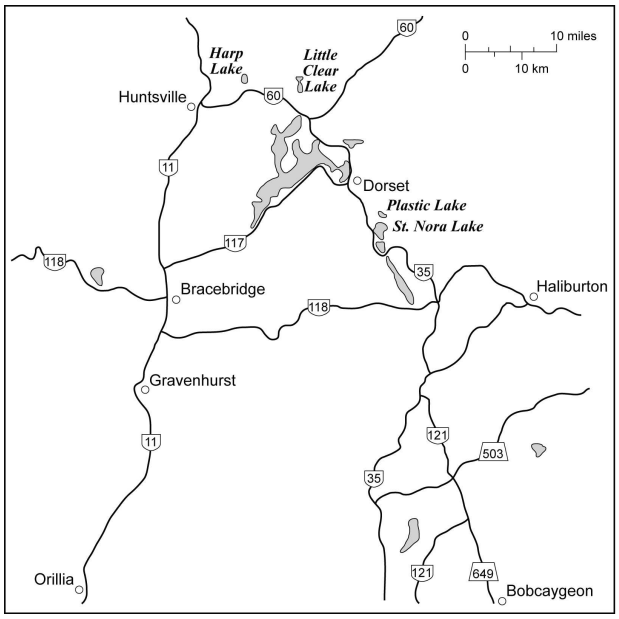
limbatum and *P. wisconsinense* are only present in lakes with pH levels ranging from 5.5 to 6.7 (Table 1.2). Another important factor shown in Table 1.3, but not focused on by Zippi et al. (1990 & 1991) is TP, which tends to co-vary inversely with pH in lakes in this region, ultimately because the two are associated with anthropogenic impact, primarily cottage development (Hall and Smol, 1996).

Table 1.2 Abundance of dinocysts (*Peridinium limbatum*- "*limb*", *Peridinium wisconsinese*- "*wisc*", *Peridinium bipes*- "*bipes*", *Peridinium willei*- "*willei*",) and other palynomorphs (the colonial green algae *Pediastrum* and zygospores= *Zygs*) in surface sediments collected during the fall of 1989 from small lakes in Haliburton-Muskoka, modified from Zippi et al. (1991). AA= very abundant, A= abundant, C= common, P=present. Locations are shown in Text-Figure 1.7. Limnological data [pH, maximum depth (*Zmax*), area in hectares [A(h)], dissolved organic carbon (DOC) and total phosphorus (TP)] are from Hall and Smol (1996).

Lake	pH	*Zmax*	A(h)	DOC	TP	*limb*	*wisc*	*bipes*	*willei*	*Pedia*	*Zygs*
Plastic	5.7	16.3	32	2.12	4.81	AA	C	C	C		
St. Nora	6.34	39	264	2.97	3.75	C	C	C	P		
Harp	6.36	37.5	542	3.68	6.33				C	C	P
Little Clear	6.82	25	149	2.77	10.53				C	A	

Table 1.3 Comparison of water quality data in Plastic and Harp Lake. Measurements of pH, dissolved organic carbon (DOC), and total phosphorus (TP) from 1980 and 2003 were taken from Yan et al. (2008), while 1994 measurements of these water quality parameters and conductivity, total nitrogen (TN) and Ca + Mg, together with the number of coastal dwellings and the assessment of the major environmental stresses (A= acidification, P= phosphorus, and NIS= non-indigenous species) were taken from Hall and Smol (1996).

Lake	pH1980 pH1994 pH2003	DOC1980 DOC1994 DOC2003	TP1980 TP1994 TP2003	Cond	TN	Ca + Mg	*Dwellings*	Stress
Plastic	5.80 5.72 5.77	2.85 2.12 2.25	8.15 4.81 4.51	22.17	174.18	2.36	0	A
Harp	6.21 6.36 6.42	4.02 3.68 3.92	9.02 6.33 6.35	37.43	220.52	3.88	83	P,NIS



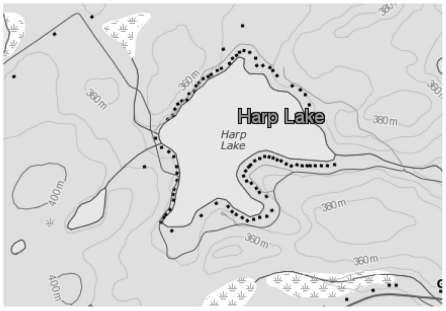


Figure 4B Harp Lake

Figure 4C Plastic Lake

Figure 1.7 Locations of the lakes sampled by Zippi et al. (1991) and whose water quality data are depicted in Table 3 are shown. Land use around Harp Lake and Plastic Lake (see topographic sheet redrawn from Natural Resources Canada Sheets 31E2 and 31E6 (http://atlas.nrcan.gc.caenglish/maps/topo/map, March 1, 2010). The difference in cottage development (squares = cottages) around these lakes (Hall and Smol, 1996) is reflected in the water quality (see Table 1.3).

The favouring of *P. willei* over *P. wisconsinense* at times of increased nutrient flux is consistent with the ecology of these dinoflagellates. *P. willei* occurs in a broad range of pH (Olrick, 1992, Niesel et al., 2007) and nutrient conditions, from eutrophic (Meyer, 1969; Pollingher et al., 1993; Anneville et al., 2002) to oligotrophic (Ternej and Tomec, 2005), in pools, lakes and peat bogs (Popovsky and Pfiester, 1990), including Lake Huron (Brown and Manney, 1983). *P. wisconsinense*, in contrast, has only been reported from mesotrophic (Meyer, 1969) to oligotrophic (Canion and Ochs, 2005) ponds and lakes with pH 6 – 7 (Phillips and Whitford, 1959; Nichols et al., 1992), including Lake Huron (Nichols et al., 1977).

1.6 Crawford Lake- an ideal setting for studies of dinoflagellates response to cultural eutrophication

The exceptional preservation in varved sediments from Crawford Lake (McCarthy and Krueger, in press; Krueger et al., 2011) provides unprecedented insights into the phytoplankton response to the two distinct phases of anthopogenic impact documented during the last millennium (McAndrews and Boyko-Diakonow, 1989), including the preservation of thecae as well as cysts at times of further depressed bottom water DO and rapid sedimentation induced by cultural eutrophication (see Chapters 3 & 4).

Crawford Lake (43°28'N, 79°57'W) is situated 278 m above sea level near the edge of the Niagara Escarpment ~50 km west of Toronto (Figure 1.8). The climate around Crawford Lake is humid continental with warm summers and cold winters (Bryson and Hare, 1974; Rowe, 1972). At nearby Millgrove Climate Station the mean annual precipitation is 973 mm, a mean temperature for July of 20.6ºC, and for January of -6ºC (Environment Canada, 2011).

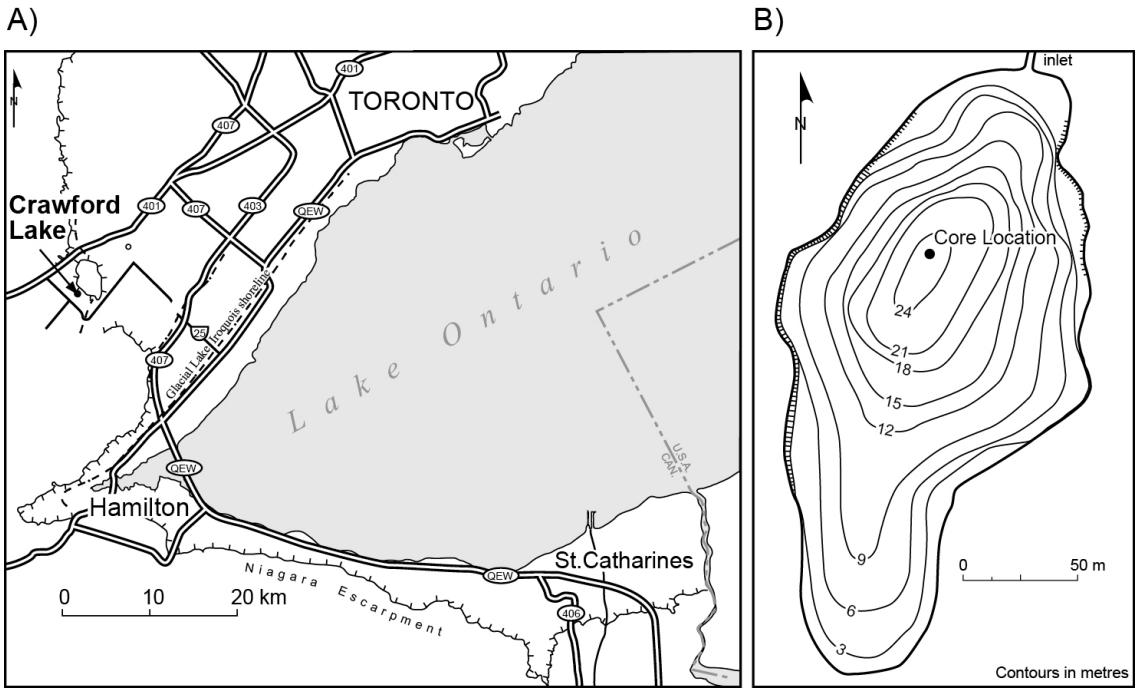


Fig 1.8 a) Location of Crawford Lake in Southern Ontario and b) contoured bathymetry of Crawford Lake (modified from Yu et al., 1997), showing the location of the core recovered in January, 2011. Hatching represents the ~6 m dolomite cliffs that partially surround Crawford Lake.

Crawford Lake lies near the boundary between deciduous forest and the Great Lakes – St. Lawrence mixed forest. Common tree taxa in this region are sugar maple (*Acer saccharum)*, white birch (*Betula papyrifera)*, beech (*Fagus grandifolia), w*hite ash *(Fraxinus Americana)*, ironwood (*Ostrya virginiana)*, white pine (*Pinus strobus)*, red oak (*Quercus rubra), e*astern white cedar *(Thuja occidentalis)*, basswood (*Tilia americana)*, hemlock (*Tsuga canadensis) a*nd white elm (*Ulmus americana)* (Rowe, 1972; Yu, 2003). Dolomite cliffs, ~6m above the lake surface, surround the northern part of the lake covered with an old growth eastern white cedar (*Thuja occidentalis*) forest. The southeastern shore of the lake is occupied by a cedar swamp (Yu, 1997).

The small (2.4 ha) lake occupies a deep dolomite basin. The Crawford lake basin is thought to have been excavated by hydraulic mining (McAndrews and Boyko-Diakonow, 1989), when glacial meltwater flowed southward between two ablating ice lobes. The raging meltwater eroded all surfical glacial deposits, leaving large slabs of Silurian bedrock (Lockport Formation.) on the edge of the escarpment, where a series of bedrock basins were excavated in the relatively soft and soluble dolomite. This process could have been further facilitated by underground caves in this karstic region.

Unlike most lakes at mid-latitudes, it does not fully overturn in fall or spring due to the relatively small surface area (~250 x 150 m) and great depth (up to 24 m of water overlies ~4.5 m of post glacial sediments in the deepest part of the basin) (Turton & McAndrews, 2006: Yu, 2003). Wind protection is further enhanced by the 6m high dolomite cliffs and old growth eastern white cedar forest that surround the lake, so the upper surface waters do not mix with the bottom waters. Meromictic conditions result, with water below 15 m lacking oxygen and having a constant temperature of 5-6 ºC (McAndrews and Turton, 2010).

Meromixis allowed annually laminated sediments to accumulate over the past ca. 2000 years (Yu, 2003). The laminations form in couplets of a white calcite-rich layer (deposited in spring/ summer) and a dark organic rich layer (deposited in late fall) (Dickman, 1979). During the fall, wind mixing of the mixolimnion results in the introduction of oxygen into the chemocline (below 15 m) resulting in a mass mortality of photosynthetic bacteria. The bacteria release sulphur as they are sinking and decomposing that reacts with the ferrous ions in the chemocline to produce black ferrous sulphides and pyrite resulting in dark organic rich lamina deposited each fall. In late spring and summer when water temperatures are at their highest calcite, starts to precipitate out (relatively abundant due to the surrounding dolomite cliffs) depositing a white calcium-rich lamina (Dickman, 1979). The anoxic bottom waters are unable to support benthic organisms, thus the lamina deposited each year are well preserved due to a lack of bioturbation (Figure 1.9).

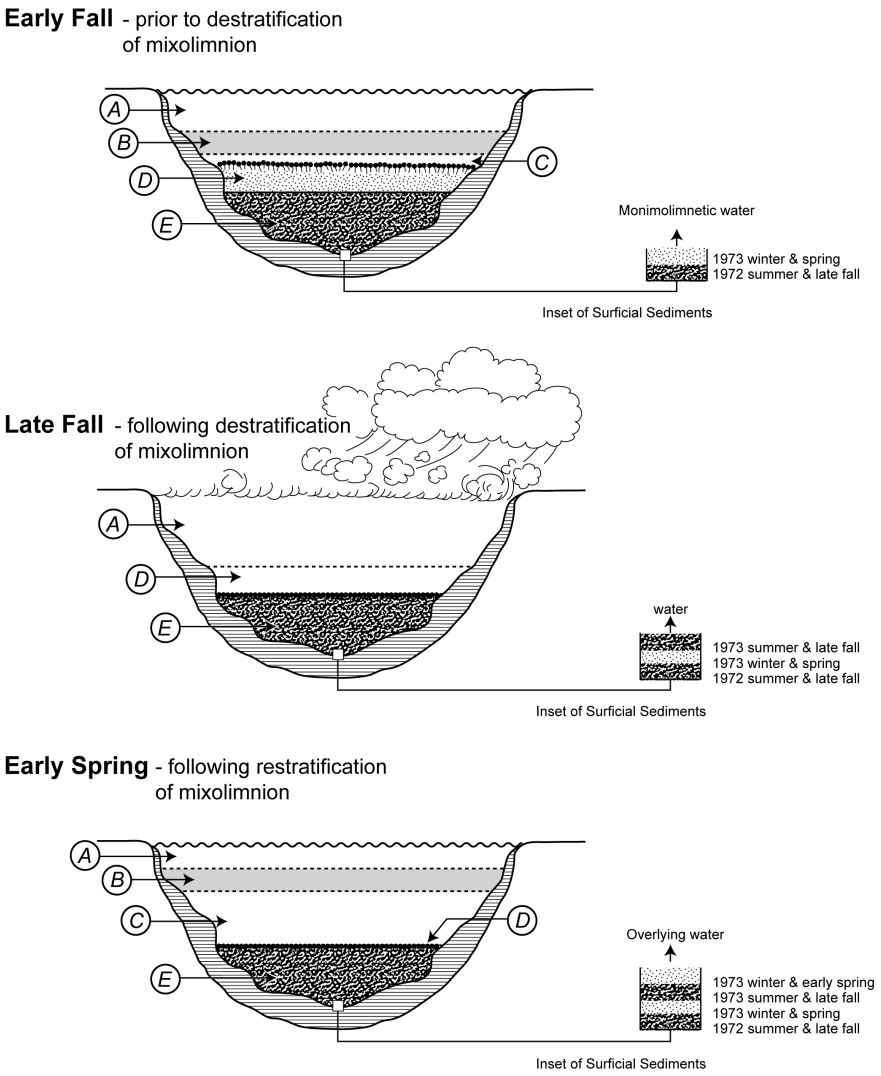


Fig. 1.9 Schematic illustration of the major seasonal changes in the water of a hypothetical meromictic lake, with insets depicting the formation of light and dark laminae. (A) Upper mixolimnion;(B) thermocline; (C) lower mixolimnion; (D) lower mixolimnion; (E) monimolimnion (from Dickman, 1979).

These varves are extremely well-preserved because there is no oxygen in the bottom waters to support bioturbating organisms that usually destroy them (Boyko, 1973; Dickman, 1979) (Figure 1.10).They allow for exceptionally accurate reconstruction of past human activity in and around the lake (with potential annual resolution) (Boyko, 1973; Boyko-Diakonow, 1979; McAndrews and Boyko-Diakonow, 1989; Zippi et al., 1990 & 1991; Ekdahl et al., 2004 & 2007; Turton and McAndrews, 2006; McAndrews and Turton, 2010; McCarthy and Krueger, in press), and the well-preserved microfossil record allows the limnological responses of the natural ecosystem to anthropogenic influence to be assessed.

Fig. 1.10 Photograph of a core from Crawford Lake revealing the well preserved annual laminae with corresponding timeline of human activity in the area (photo courtesy C. Turton).

Two distinct periods of human habitation have been identified from the pollen record in Crawford Lake (Figure 1.11). Pollen of *Zea mays*, along with *Cucurbita*, *Portulaca* and *Helianthus* in sediments deposited between ca. A.D. 1268 and 1486 (estimated from varve counts) record Iroquoian agriculture (Boyko, 1973; Byrne and McAndrews, 1975; Byrne, 1998). The area was subsequently uninhabited until the early 1800s when Euro-Canadian farmers cleared forests for wheat fields. Because the very thin soils and rocky outcrops immediately around Crawford Lake were unfavourable for farming, the slopes around the lake remained unsettled until A.D 1883, when George Crawford purchased 100 acres of land and built a lumber mill at the south end of the lake. The mill supplied cedar shingles to the surrounding community for housing, but the primary product was white pine and hemlock lumber shipped all over southern Ontario and used in the construction of the Welland Canal. The Crawfords built a cottage on the edge of the lake in 1889 and stocked the lake with four types of fish: speckled trout

(*Salvelinus fontinalis)*, lake trout (*Salvelinus namaycush*), rainbow trout (*Oncorhynchus mykiss*) and small mouth black bass (*Micropterus dolomieu*) (the bass are all that remain today of the introduced fish species). In 1969, the Crawfords sold the land to Conservation Halton in order to preserve the lake (Crawford Lake Conservation Area, 2011). The discovery of pollen of cultivars in varved sediments led to the archaeological excavation and re-creation of the village, and the construction of the visitor's centre in the 1970's (Boyko, 1973).

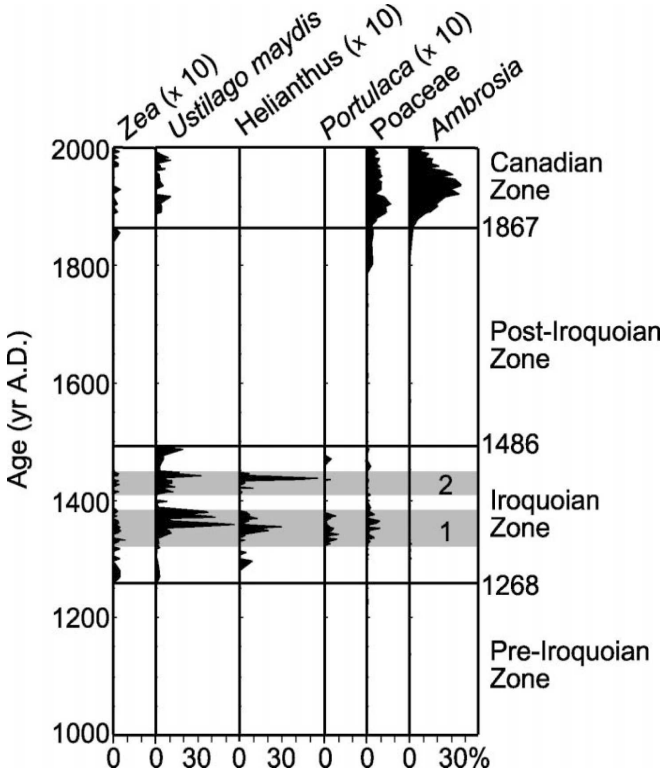


Fig. 1.11 Ekdahl et al. (2004) summarized palynological evidence of human impact around Crawford Lake. The presence of pollen of cultivars (*Zea*- corn, *Helianthus*- sunflower, *Portulaca*- purslane), and of parasites associated with some of these cultivars (e.g. *Ustilago maydis*- corn smut) identifies Iroquoian agriculture near Crawford Lake between the 13th and 15th centuries. Abundant non-arboreal (herb) pollen, such as grasses (Poaceae) and ragweed (*Ambrosia*) records deforestation associated with Euro-Canadian colonists in the 19th century. Units in percent of total counted pollen grains.

The availability of published literature investigating the response of multiple proxies to the well-documented periods of human impact, separated by centuries of regeneration in the lake and in the watershed (Boyko, 1973; Boyko-Diakonow, 1979; McAndrews and Boyko-Diakonow, 1989; Zippi et al., 1990 & 1991; Ekdahl et al., 2004 & 2007; Turton and McAndrews, 2006; McAndrews and Turton, 2010; McCarthy and Krueger, in press)

makes Crawford Lake an ideal location to further investigate the potential of dinoflagellate cysts as proxies of cultural eutrophication.

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Chapter 2: Freshwater dinoflagellates in paleolimnological studies: as proxies of cultural eutrophication in the southeastern Great Lakes region of Ontario, Canada

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2.1 Introduction

Recent studies illustrate the potential of dinoflagellates as paleolimnological proxies using a combined phycological and palynological approach, relating the stratigraphic and biogeographic distribution of cysts attributed to several species of *Peridinium* to environmental conditions in lakes with documented anthropogenic impact. Cultural eutrophication associated with both indigenous and Euro-Canadian settlement at various sites in the Great Lakes region of Ontario is expressed by a sharp increase in dinocyst abundance and a decline in *Peridinium wisconsinense* Eddy relative to species better adapted to eutrophic conditions. Total concentrations exceeded 46,000 cysts per ml in varved sediments deposited when Crawford Lake was impacted by Iroquois agriculture in the 13th - 15th C, even higher than the peak concentrations of nearly 36,000 cysts per ml associated with Euro-Canadian logging and agriculture. Most of these cysts were attributed to *Parvodinium inconspicuum* (Lemmermann) Carty and *Peridinium volzii* Lemmermann*,* whose cysts have not previously been reported in other palynological studies from this region. The lack of reports of these cysts elsewhere may reflect exceptional preservation of cysts and thecae in Crawford Lake, or the failure of palynologists to notice these relatively small, nondescript cysts and recognize their affinity.

The armoured dinoflagellate genus *Peridinium* Ehrenberg (Order Peridiniales Haeckel, Family Peridiniaceae Ehrenberg) is common in freshwater environments throughout the Great Lakes region of North America (Prescott 1962; Nicholls *et al*. 1992; Carty 1993, 2002; McCarthy *et al*. 2011). Thecae range from ~10-100 µm in length, and contain ~20 plates with tabulation 4', 2-3a, 5-6c, 7'', 5''',2'''' (Hansen & Flaim 2007) (Fig. 2.1). Plate arrangement and ornamentation is used to identify dinoflagellates to species (Carty 2002), but phycological surveys generally identify most taxa only to genus, identifying only a few very distinctive species, e.g. *Peridinium wisconsinense* Eddy and

Parvodinium inconspicuum (Lemmermann) Carty (Nicholls *et al*. 1992; McCarthy *et al*. 2011). The identification of thecae is not straightforward because a wide range of morphotypes can occur within species (Popovsky and Pfiester, 1990). Kim *et al*. (2004), for instance, found greater genetic variation between the two populations of *Peridinium limbatum* (Stokes) Lemmermann within a single drainage basin in Wisconsin than that reported in the literature for some morphologically distinguishable microalgal species.

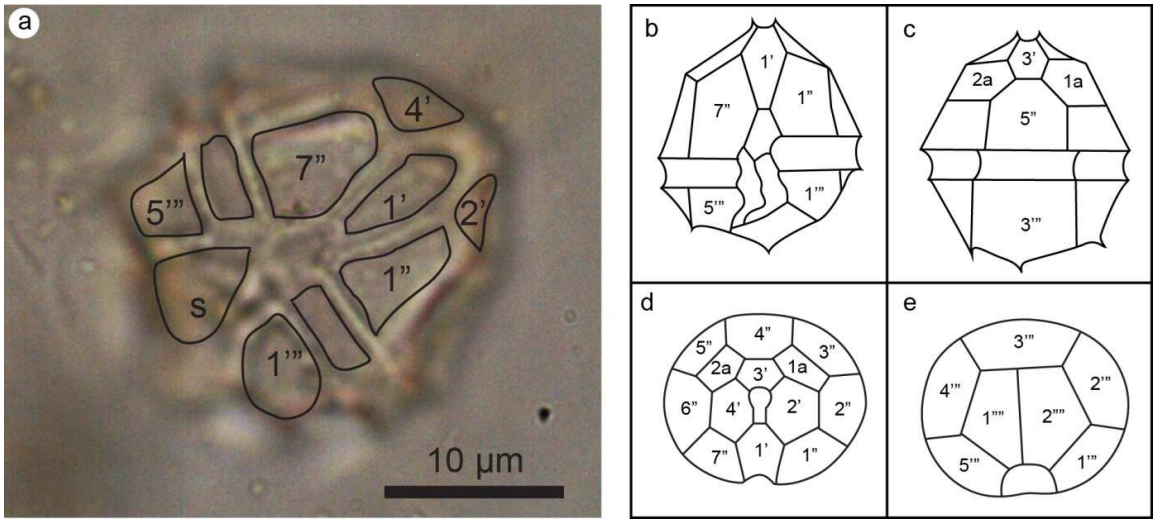

Fig.2.1 (a) Micrograph of a theca of *Parvodinium inconspicuum* (Lemmermann) Carty exceptionally preserved in palynological preparations from varved sediments in Crawford Lake illustrating the tabulation. Schematic drawings of *P. inconspicuum* (Lemmerman) Carty showing the tabulation from b) ventral, c) dorsal, d) apical and e) antapical perspectives (modified from Shen et al., 1990).

Theca-cyst relationships also remain poorly understood within the genus *Peridinium*, and it is not known how many freshwater dinoflagellate species produce fossilizable resting cysts. Norris & McAndrews (1970) tentatively attributed cysts (types A-D) in Holocene sediments from Glatsch Lake, MN to four extant species. Culturing studies (Evitt & Wall 1968; Wall & Dale 1968) and biogeography allowed them to attribute cyst types A and B to *Peridinium limbatum* (Stokes) Lemmermann and *Peridinium wisconsinense* Eddy, but they inferred the affinity of the other two common cyst types to *Peridinium bipes* Stein and *Peridinium willei* Huitfeld-Kaas with less confidence, on the basis of gross morphological similarities. These theca-cyst relationships were subsequently employed in studies of Holocene lake sediments from Ontario (Fig. 2.2) by Burden *et al*. (1986) and Zippi *et al.* (1991). The biogeographic distribution of *Peridinium bipes* Stein made it unlikely that its cysts were present in sediments from Glatsch Lake, however, and culturing and DNA sequencing by McCarthy

et al. (2011) suggests that both types C and D of Norris & McAndrews (1970) can be attributed to *Peridinium willei* Huitfeld-Kaas.

Little work has been done on the ecology of freshwater dinoflagellates, and even less has been done on the biogeographic distribution and taphonomy of their resting cysts, but over the last few decades a number of palynological investigations have identified cysts attributed to various species of *Peridinium* and attempted to relate their stratigraphic and biogeographic distribution to environmental conditions, most notably in the Great Lakes region of Ontario (Fig. 2.2). Even in studies where both phycological and palynological approaches are combined, it has proven difficult to definitively relate thecae with cysts. Tardio *et al.* (2006), for instance, were unable to relate the three peridinioid cyst morphotypes in sediments from Lake Nero de Cornisello, Italy to thecae present in the water column- *Peridinium cinctum* (Muller) Ehrenberg and *Peridinium umbonatum* Stein. Similarly, Chu *et al.* (2008) attempted to relate the cysts making up sedimentary laminations to one of the two species of dinoflagellates found in the water column of Lake Xiaolongwan, China (*Peridinium volzii* Lemmermann and *Ceratium furcoides* (Levander) Langhans), but instead attributed the dark brown pyriform to ovoid cysts to *Parvodinium inconspicuum* (Lemmermann) Carty based primarily on the small size of the cysts. The dark brown pyriform cysts in Lake Xiaolongwan are similar to cysts identified by Tardio *et al.* (2006) in Lake Nero, but quite different from tiny, unornamented spherical cysts attributed to *P. inconspicuum* (Lemmermann) Carty from Crawford Lake, however (Fig.. 2.1, Photo Plate 2.1) (Krueger et al., 2011).

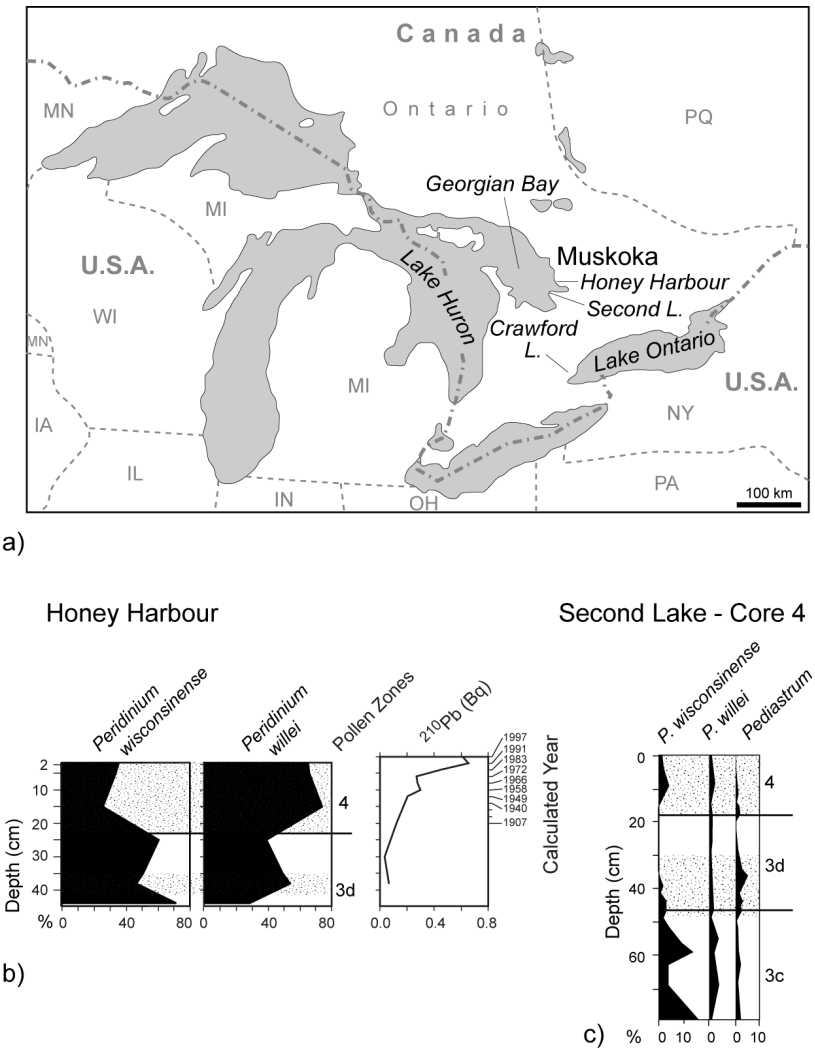

Fig. 2.2 (a) Location of sites in the southeastern Great Lakes region of North America where dinocyst distribution has been related to ecological parameters, notably anthropogenic impact (Second Lake- Burden et al., 1986; Honey Harbour- McCarthy et al., 2011; Crawford Lake- Krueger et al., 2011). Zippi et al. (1991) studied the distribution of dinocysts in surface sediments from various lakes in the Muskoka region of Ontario, east of Georgian Bay. (b) Stippling identifies core horizons with evidence of Wendat (Huron) and Euro-Canadian impact on the drainage basins of Honey Harbour and (c) Second Lake (now in a Provincial Park).

2.2 Dinocysts in laminated sediments from Crawford Lake

The annual laminations that have accumulated in the meromictic Crawford Lake over the last several millennia offer an unprecedented opportunity to investigate the potential of dinocysts as paleolimnological indicators, particularly for studies of cultural eutrophication (Krueger *et al.* 2011). Evidence of Iroquois agriculture in the 13th – 15th century and Euro-Canadian logging and agriculture beginning in the mid 19th century was first documented in the pollen record (Boyko 1973, Boyko-Diakonow 1979;

McAndrews & Boyko-Diakonow 1989).The impact of these two phases of human settlement on the ecology of this small lake has been documented in studies of diatoms (Ekdahl *et al*. 2004, 2007) and rotifers (Turton & McAndrews 2006). Varves with very high total concentrations of cysts, many with distinctive red bodies (peaking at ~46,000 cysts per ml), and preserved thecae, were deposited during Iroquois (~AD 1268 to 1486) and Euro-Canadian (since ~AD 1867) settlement of the drainage basin (Fig. 2.3) (McAndrews and Boyko-Diakonow, 1989; Ekdahl *et al*., 2004). High total cyst concentrations were also associated with both Wendat ("Huron") settlement of the Penetanguishene Peninsula (~A.D. 1450-1650; Heidenreich 1971 in O'Brien 1976) and Euro-Canadian settlement of the region (since ~A.D. 1840; Chittenden 1990). Total cyst concentrations peaked around 14,000 and 12,000 cysts per ml in Honey Harbour, during the two phases of human impact (McCarthy et al. 2011), substantially lower than values estimated for Crawford Lake.

Four distinct cyst morphotypes (Photo Plate 2.1) in varved sediments from Crawford Lake have been attributed to *Peridinium wisconsinense* Eddy, *Peridinium willei* Huitfeld-Kaas, *Peridinium volzii* Lemmermann, and *Parvodinium inconspicuum* (Lemmermann) Carty. These dinoflagellate taxa have been reported in various phycological investigations from lakes in northeastern North America (e.g. Nicholls et al. 1992; Carty 1993, 2002), and cyst-theca relationships have been demonstrated for *Peridinium wisconsinense* Eddy by Wall & Dale (1968) and McCarthy *et al*. (2011), for *Peridinium willei* Huitfeld-Kaas by McCarthy *et al*. (2011) Although cyst-theca relationships have not previously been established for *Parvodinium inconspicuum* (Lemmermann) Carty and *Peridinium volzii* Lemmermann*,* thecae exceptionally preserved in palynological preparations rich in these cysts (Fig. 2.1, Photo Plate 2.1) from varved sediments from this small, deep lake north of Lake Ontario (Fig. 2.1), make their affinity difficult to refute.

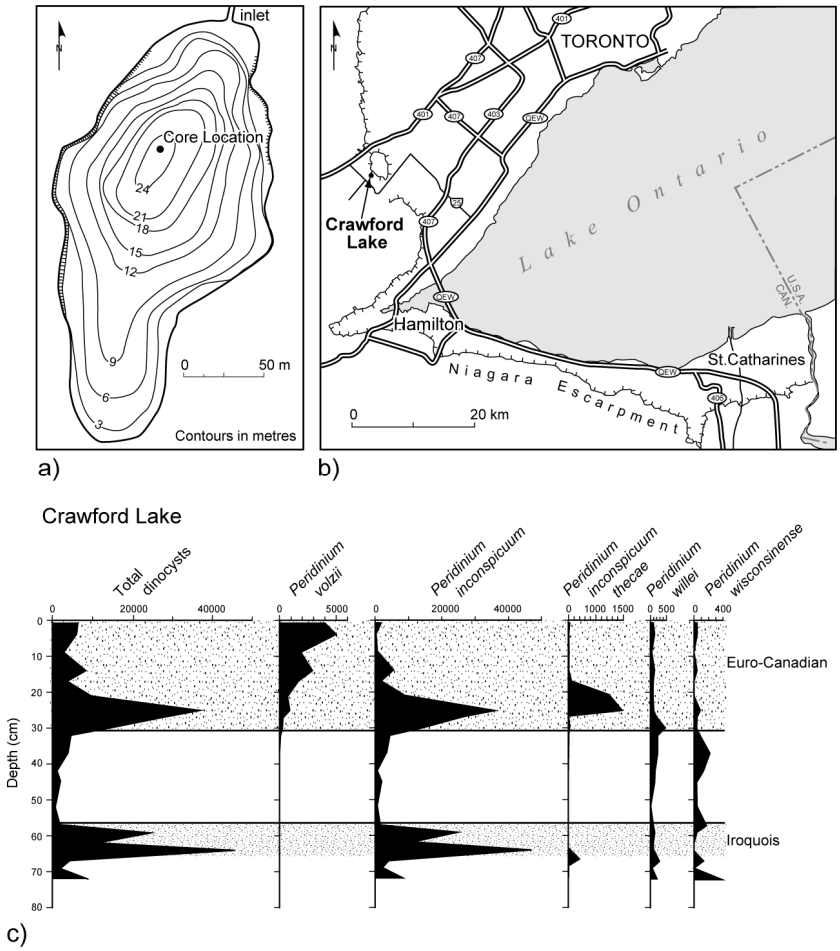

Fig. 2.3 (a) The small, deep, meromictic Crawford Lake basin (b) on the edge of the Niagara Escarpment near Toronto (c) contain very high cyst concentrations as well as exceptionally preserved thecae (most notably of the tiny *Peridinium inconspicuum* Lemmerman) in varves dating to intervals of Iroquois and Euro-Canadian settlement (stippled). Cysts attributed to *Peridinium volzii* Lemmerman are common only in sediments deposited during Euro-Canadian settlement. Peak abundances of *Peridinium wisconsinense* Eddy at times of negligible human impact is consistent with observations in other lakes in the Great Lakes region (see Fig. 2.2).

In contrast, total cyst concentrations range from ~400 to 4000 cysts per ml (average ~1500 cysts per ml) in sediments deposited in Crawford Lake between AD ~1486-1867, reflecting much lower nutrient flux (Fig. 2.3). Sparse assemblages prior to Iroquois impact and between the two phases of human settlement in Crawford Lake were dominated by relatively large cavate, proximate cysts *Peridinium wisconsinense* Eddy, easily identified from their rounded, sometimes bifurcated apical horn and a single, sharply pointed antapical horn (Photo Plate 2.1). These cysts also dominated the assemblage in Second Lake and in Honey Harbour prior to Wendat settlement, and they

increased in relative abundance in Honey Harbour between the two phases of settlement in the Penetanguishene Peninsula (Fig. 2.2). The association of cysts of *P. wisconsinense* with low nutrient flux is consistent with the ecology of these dinoflagellates that have been reported only from mesotrophic (Meyer 1969) to oligotrophic (Canion & Ochs 2005) ponds and lakes in North America. Reports of the occurrence of this taxon in the water column are considered reliable due to its easily recognisable and distinctive morphology, allowing even non-specialists to identify it to species in phytoplankton surveys (e.g. Nicholls *et al*. 1992; McCarthy *et al*. 2011). Zippi *et al*. (1991) also found cysts of *P. wisconsinense* in surface sediments from only a few of the 11 lakes they studied, restricted to lakes with low total phosphorus concentrations (TP<5 µg/L; Hall & Smol, 1996). They found cysts of *P. willei* Huitfeld-Kaas to be ubiquitous, in contrast, and especially abundant in lakes rich in other algal microfossils associated with eutrophic conditions [e.g. *Pediastrum* spp., and zygnematacean zygospores (desmids)].

Although tiny (~16-20 µm diameter) unornamented spherical cysts attributed to *Parvodinium inconspicuum* (Lemmermann) Carty are abundant in sediments associated with both phases of human settlement in Crawford Lake, larger (~30 by 40 µm) slightly ovoid cysts attributed to *Peridinium volzii* Lemmermann (Photo Plate 2.1) are abundant only in varves deposited since ~AD 1880. The abundance of cysts attributed to these taxa in sediments impacted by humans is consistent with their ecology and biogeography, but cysts have not previously been reported in sediments from North American lakes. While this could reflect the failure of palynologists to notice these small, relatively nondescript cysts in their slides and attribute them to dinoflagellates, it could also reflect the very different taphonomic environment in the anoxic bottom waters of Crawford Lake, allowing thecae with viable cell contents to survive for centuries (Krueger *et al*., 2011).

Although dinocyst taphonomy has received little attention, McCarthy *et al*. (2011) attributed the high concentrations and extraordinary preservation of the membranes of cysts in the H_2S -rich black, gelatinous gyttja deposited in the seasonally anoxic waters of Honey Harbour to taphonomic factors, relative to the cyst assemblages in the rest of Severn Sound, which is subject to strong bottom currents. Dinocyst concentrations during times of anthropogenic impact by both indigenous and Euro-Canadian settlers were more than three times higher in the varves from Crawford Lake than they were in gelatinous gyttja from Honey Harbour. This, together with the exceptional preservation of

thecae (Fig. 2.3, Photo Plate 2.1) in dinocyst-rich varves associated with cultural eutrophication, suggests even better preservation in the anoxic bottom waters of the meromictic Crawford Lake than in the seasonally anoxic lakebed in Honey Harbour, so care should be taken in extrapolating from this lagerstatte to other North American lakes. Strong taphonomic control of dinocyst distribution is also suggested by the sparse dinocyst record in recent sediments from Cook's Bay, despite the abundance of other algal microfossils in these marly muds deposited at times of very high nutrient flux, suggesting poor preservation of dinosporin in alkaline conditions (Danesh, 2011).

Further phycological and palynological work on this largely ignored microfossil group is encouraged in order to better understand the relationships between cysts preserved in the sediment and conditions in the water column. Dinoflagellate cysts are found in slides processed for pollen analysis, which remains the best proxy of human impact in the watershed, so their identification by palynologists would prove useful in reconstructing pre- and post- impact conditions in the water bodies where the pollen accumulates. Dinocysts could be valuable in the management of lakes, since the response of critical freshwater resources to environmental perturbations (both natural and anthropogenic) can be assessed using time series of environmental data recorded by microfossils preserved in lake sediments in a way that synoptic measurements of water quality cannot. Thus they appear to have the potential to be a useful and efficient tool in studies of eutrophication, as they have in marine environments (Dale *et al*. 1999; Dale, 2009).

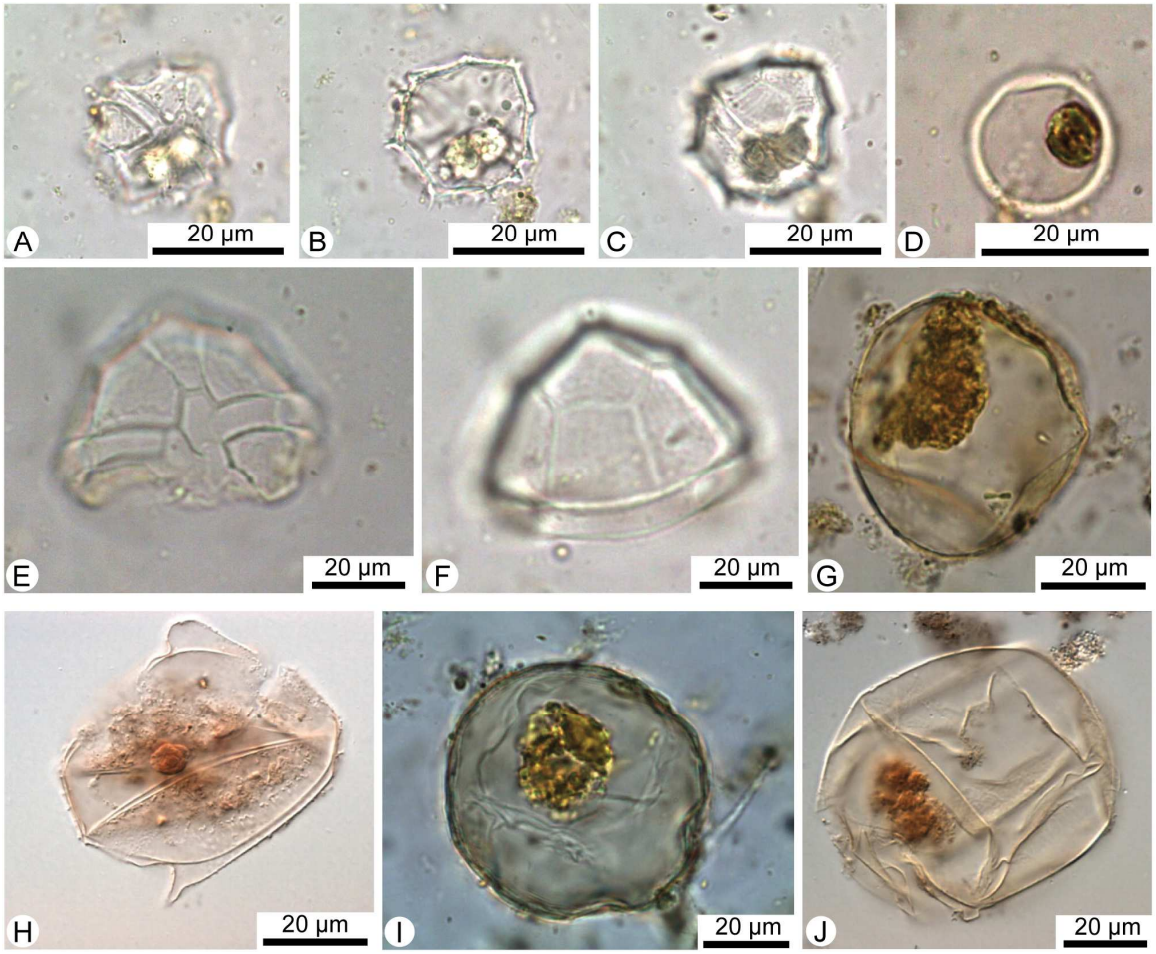

Photo Plate 2.1 A-C Ventral through dorsal views of small (~20-25 µm by 20-30 µm) thecae of *Peridinium inconspicuum* Lemmermann, showing the pronounced apical horn and scattered antapical spines. These are preserved in palynological preparations of varved sediments from Crawford Lake together with (D) abundant tiny (~16-20 µm in diameter), unornamented spherical cysts, commonly containing red bodies. E,F Ventral and dorsal views of an ovoid and slightly dorso-ventrally flattened theca of *Peridinium volzii* Lemmerman, similar to *P. willei* Huitfeld-Kaas but smaller in size (~38-45 µm by ~52-60 µm), and with a considerably smaller 1'-plate. These were found in palynological preparations with (G) abundant small (~30 by 40 µm), unornamented cavate, proximate cysts with distinctive red bodies. H Cavate, proximate cyst of *Peridinium wisconsinense* Eddy, easily identified from its rounded, sometimes bifurcated apical horn and a single, sharply pointed antapical horn, measuring ~62 µm from horn to horn. (from Honey Harbour Core SV5-C). I, J Cavate, proximate cysts of *Peridinium willei* Huitfeld-Kaas, with the outer layer slightly invaginated in the sulcal area to form two distinct shoulders that together with the larger size (~50 by 60 µm) distinguish it from cysts of *P. volzii* Lemmerman (I from Honey Harbour Core SV5-C, J from Crawford Lake core).

2.3 Acknowledgements

We are grateful to C. Turton and J.H McAndrews for their assistance with collecting and interpreting the freeze core, to M.J. Head for his taxonomic insights, and to M. Lozon for assistance with drafting. We also acknowledge the organisers and editors of this special volume and the delegates of DINO9 who provided feedback and encouragement, and the reviewers whose suggestions improved the manuscript.

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Chapter 3: Exceptionally preserved dinoflagellates in varved Holocene sediments from Crawford Lake, Ontario, Canada.

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In preparation for Journal of Paleontology

3.1 INTRODUCTION

Thecae of the freshwater dinoflagellate *Parvodinium inconspicuum* (Lemmerman) Carty were preserved in varved sediments from 67 cm and from 25 through 22 cm in a freeze core from Crawford Lake, a meromictic lake near Toronto. The presence of these thecae allowed the affinity of abundant tiny (~16-20 µm in diameter), smooth, spherical to slightly ovoid cysts with golden-brown plastids in the same palynological preparations to be determined. Varve counting suggests that this exceptional preservation occurred when the lake was impacted by Iroquois, who practiced agriculture in the region during the 13th to 14th century, and Euro-Canadians, who settled, logged, and farmed the land around the lake beginning in the mid 19thC. Cultural eutrophication apparently increased productivity and sedimentation rates, further depleting bottom waters of this small, deep lake of dissolved oxygen (DO). Other cysts found in low abundances throughout the 72 cm freeze core were assigned to *Peridinium wisconsinense* Eddy and *Peridinium willei* Huitfeld-Kaas based on previous culturing and rDNA studies of these cyst types from other lakes in the region.

Cell contents are visible in cysts through most of the core, and cysts remained viable for over a century with inadvertent culturing of samples down to 29 cm (~A.D. 1845-1860). Spongy-walled hypnozygotes of *P. inconspicuum* were observed undergoing meiosis, and their similarity to the tiny resting cysts found in the sediments reaffirms the inferred theca-cyst affinity. Larger (~38-45 µm by ~42-50 µm) cavate and proximate cysts lacking ornamentation or shoulders were also observed encysting, and resulting empty thecae resemble *Peridinium willei* Huitfeld-Kaas. They are smaller than thecae of *P. willei* previously documented in lakes in this region, are ovoid and slightly dorso-ventrally flattened, and have a considerably smaller 5-sided1'-plate, allowing us to identify them as *Peridinium volzii* Lemmerman. Their cysts are impossible to distinguish

from cysts of *P. willei* except by size, and are restricted to the upper 32 cm of the core, i.e. since the mid 19th century.

"Armored" dinoflagellates in the Order Peridinales Haeckel have thecae made of plates of a cellulose-like complex polysaccharide. An equatorial groove in the thecal plates (the cingulum) holds the transverse flagellum and a posteriorly-directed groove (the sulcus) holds the longitudinal flagellum, resulting in a corkscrew motion as these protists propel themselves through the water. The epitheca is anterior to the girdle, the hypotheca is posterior to the girdle (Fig. 3.1). Plastids are golden-brown in color, becoming bright red when the cells are about to undergo mitosis or encyst, as was noted by Nicholls (1973).

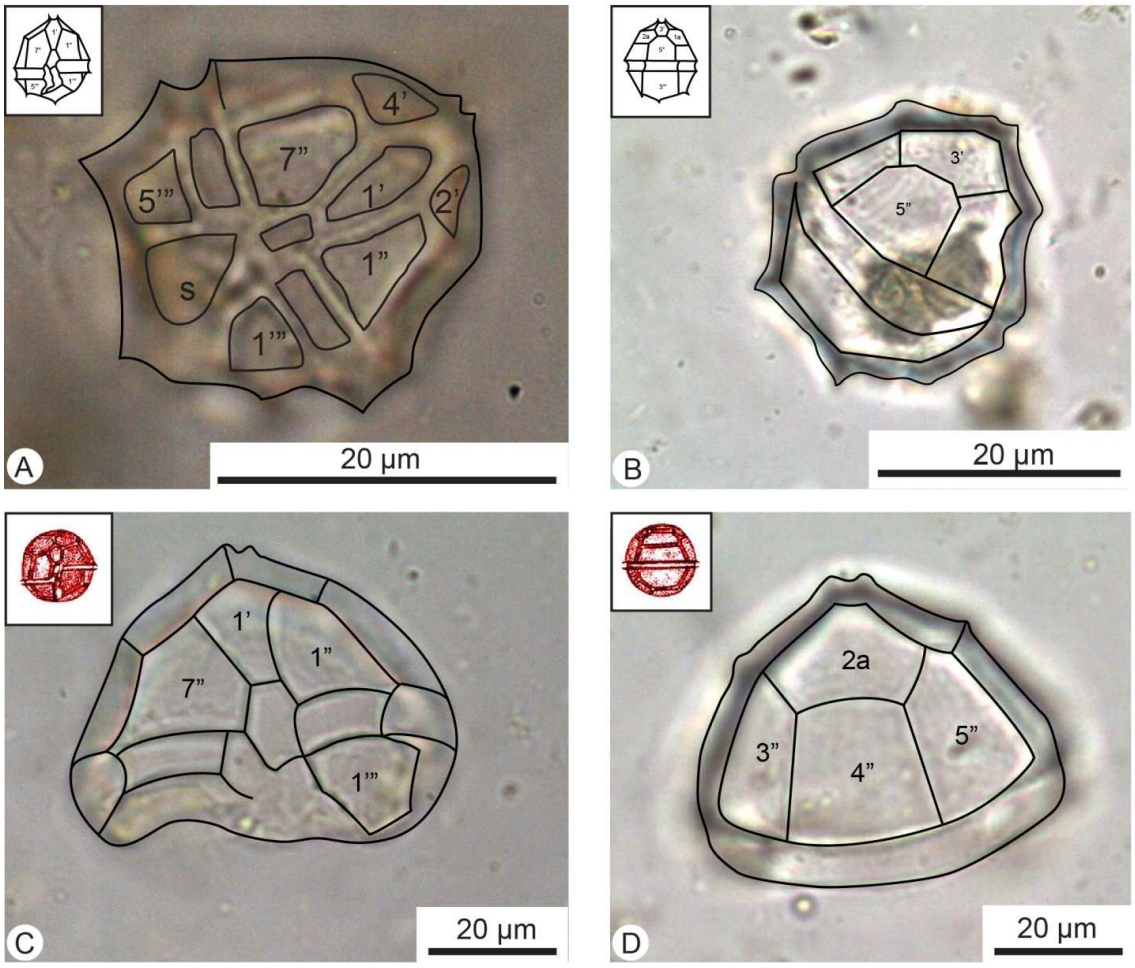

Figure 3.1 (A & B) Micrographs of the ventral and dorsal perspectives of *Parvodinium inconspicuum* (Lemmermann) Carty and (C & D) *Peridinium volzii* Lemmerman thecae exceptionally preserved in palynological preparations from varved sediments in Crawford Lake illustrating the tabulation. (Insets of schematic drawings modified from Shen et al., 1990.)

Thecate cells of the Family Peridiniaceae Ehrenberg contain approximately 20 thecal plates, with 5 postcingular and 2 antapical plates (e.g. Popovsky and Pfiester 1990). The majority of fossilized cysts in freshwater sediments have been attributed to the genus *Peridinium* Ehrenberg. The identification of this genus and its many species that range in size from ca.10 µm to nearly 100 µm in length is not without challenges (Popovsky, 1983). It relies on the arrangement of thecal plates ("plate tabulation": 4', 2-3a, 5-6c, 7'', 5''',2'''') and the ornamentation of these plates (Hansen and Flaim, 2007). Recent studies based on molecular analysis small subunit (SSU) ribosomal DNA, however, indicate that the genus *Peridinium* as presently defined is polyphyletic (e.g. Saldarriaga et al*.* 2004; Hansen and Flaim, 2007), and tremendous infraspecific variation has been found, both morphologic (Popovsky and Pfiester, 1990) and genetic (Kim et al., 2004). Carty (2008) has reassigned tiny dinoflagellates previously assigned to *Peridinium* (as *Peridinium inconspicuum* and *Peridinium umbonatum*) to the genus *Parvodinium* Carty. The Umbonatum Group differs from other *Peridinium* species in that they have an apical pore; cells are small (~12-20 µm) with two apical intercalary plates and six cingular plates. This differs from *Peridinium cinctum* Ehrenberg, the type species for this genus, that lacks an apical pore, is much larger (~40-64 µm) with three intercalary plates and five cingular plates (Carty, 2008). Molecular analysis SSU rRNA (Saldarriaga et al., 2004) and large subunit (LSU) data (Logares et al., 2007) also found that *P. cinctum, P. bipes, P. gatunense, P. volzii*, and *P. willei* could be separated from the Umbonatum Group species (*P. umbonatum, P. inconspicuum, P. centenniale*) (Carty, 2008).

Peridiniacean cysts produce two types of cyst that have thicker walls than thecae (vegetative cells): relatively short-lived hypnozygotes that participate in sexual reproduction, and longer-lived "resting cysts" of dinosporin. These resting cysts give these phytoplankton an advantage in allowing them to temporarily escape herbivory by postponing excystment until herbivores are less abundant (Rengefors and Anderson, 1998) and even to survive catastrophic events. These dinosporin cysts are typically the only dinoflagellate remains found in the fossil record, although cellulosic thecae have been reported preserved in other exceptional circumstances.

Dinoflagellates are one of the major constituents of the summer phytoplankton in freshwater environments throughout the Great Lakes region of North America (Prescott, 1962; Nicholls et al., 1992; Carty, 1993 & 2002), but their fossil record has received little attention. Traverse (1955) reported the occurrence of *Peridinium hansonianum* from

Oligocene lignites of presumed freshwater origin, and since then there have been only a dozen or so reports of cysts of *Peridinium* spp., primarily from Holocene sediments (Evitt and Wall, 1968; Wall and Dale, 1968; Norris and McAndrews, 1970; Bourrelly and Coute, 1980; Burden et al., 1986; Zippi et al., 1990, 1991; Wu and Chou, 1998; Kim et al., 2004; Wang et al, 2004; Tardio et al., 2006; Chu et al., 2008; Krueger et al, 2011, McCarthy et al., 2011; Mertens et al., in press). Their occurrence is usually noted to confirm a freshwater depositional environment or freshwater flux (Haas, 2010), but more recently they have shown potential as proxies of cultural eutrophication (Burden et al., 1986; McCarthy et al., 2011; McCarthy and Krueger, in press).

The preservation of both thecae and cysts of several species of peridiniacean dinoflagellates in a late Holocene lagerstatte provides insights into the affinity of previously unidentified cysts and into the ecology and taphonomy of cyst-forming freshwater dinoflagellates. Meromixis resulting from the physiography of Crawford Lake (Dickman, 1979) allowed varved couplets to accumulate in the oxygen-depleted monimolimnion, and exceptional preservation of several planktonic microfossil groups has been reported (e.g., Ekdahl et al., 2004; Turton and McAndrews, 2006; McAndrews and Turton, 2010; Chan et al., submitted). Preservation of non-pollen palynomorphs (Turton and McAndrews, 2006) and ostracods (Chan et al., submitted) was particularly good during the two distinct periods of anthropogenic impact whose ages are estimated from varve counts (Iroquois ~A.D. 1268 to 1486; Euro-Canadians ~A.D. 1867 to 1969) (McAndrews and Boyko-Diakonow, 1989; McAndrews and Turton, 2010).

3.2 THIS STUDY

A 72 cm-long freeze core was recovered on January 25, 2011, from the deepest point in Crawford Lake (~22.5 m) using a frigid fingernail sampler, a hollow aluminum wedge filled with an ethanol and dry ice slurry. A hole was bored through the ice in which the frigid fingernail sampler tethered to a rope was lowered by man power through the ice to the lake bottom. The wedge design of the frigid fingernail sampler allows for easy penetration into the sediments. The sampler with the frozen sediments were brought to the surface and transported to shore on a sled where with the use of warm water the core was removed from the sampler and wrapped with aluminum foil. The core was brought back to Brock University where it was kept in a freezer wrapped in dry ice awaitning further processing. Febuary, 2011 with the help of Jock McAndrews, Charlie

Turton, Laura Lee Tobicoe and Calvin Chan the core was processed for sampling. Photographs of the core were taken before and after planing and scraping the outer surface away to reveal the undisturbed frozen varves (Fig. 3.2). Annual couplets are comprised of a white varve that is very rich in calcite precipitated in relatively warm surface waters during the summer, and a dark varve that forms in the fall when mass mortality of plankton occurs. Dickman (1979) suggests that sulphur is released by photosynthetic bacteria as they decompose that reacts with ferrous ions in the chemocline to produce black ferrous sulphides and pyrite, enhancing the dark colour of the varves.

Sediments are reddish-brown at the base of the core, becoming dark brown around 65 cm, where well-defined varves first appear. Canada goose (*Branta Canadensis)* fecal pellets are present within the algal rich laminations, as they were in varves in the 2001 freeze core deposited during the Iroquois period (McAndrews and Turton, 2010). The sediments return to a reddish brown colour up-core beginning ~52 cm, reflecting at least periodic oxidation, and the varves become irregular until 45 cm, where very fine laminations persist to a depth of 30 cm. The sediments then become greenish black, reflecting an increase in organic carbon content and increasingly reducing conditions. The laminations thicken to a maximum of 1-2 mm from 18 cm – 7 cm, and then thin out again to top of the core, but annual couplets remain clearly visible (Fig. 3.2).

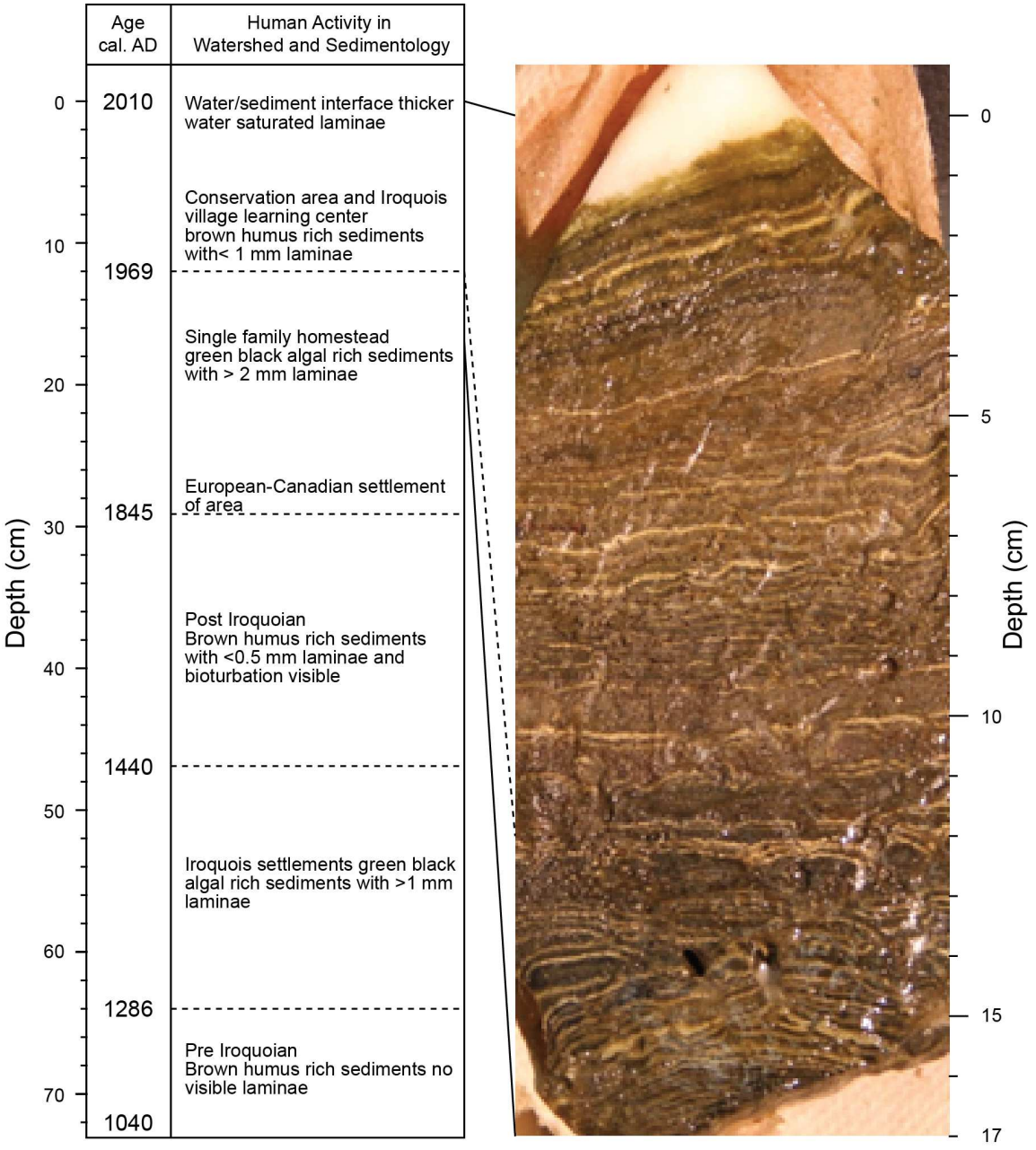

Fig. 3.2 Summary diagram outlining the history of Crawford Lake for the past ~1,000 years. Inset photo of the upper 17 cm of the freeze core retrieved in Jan 2011 from the deepest part of Crawford Lake, showing light and dark annual couplets.

The age model for the core samples in this study is based on varve counts from the top of the core (A.D. 2010) and visually correlated with the Ekdahl et al. (2004) core collected in 2001, in which varve counts were supplemented with dated with 25 AMS dates. The age model suggests that the base of the core was deposited ca. 1040 AD.

Twenty one 2.5 ml sediment samples were taken from the 72 cm-long core. Sample volumes were measured using liquid displacement, and sediments were processed using a weak (10%) HCl solution spiked with a tablet containing 10,850 +/- 200 spores of *Lycopodium clavatum* (batch # 006720). The samples were then sieved using a 15µm *Nitex* sieve and distilled water. The sample residue was mounted to slides using glycerin jelly on a hot plate. The strew slides were examined using a Leica DMLB light microscope at 400X magnification and photographed using oil immersion at 1000X magnification using a Leica EC3 Digital Imaging Camera. Counts used for downcore distribution data was based on a minimum count of 60 dinocysts per millilitre and 200 grains of pollen/ml.

Inadvertent culturing occurred when samples were left in test tubes overnight in the weak HCl solution, allowing some several century-old dinoflagellates to excyst. The various stages of their life cycles were photographed, and duplicate samples were processed to avoid erroneous counts of fossil cysts, thus reflecting dinocyst assemblages and concentrations at time of deposition.

3.3 RESULTS AND OBSERVATIONS

Cysts with distinct dinoflagellate affinity (acid-resistant walls, often with golden-brown plastids, sometimes with evidence of paratabulation) were present in all 21 samples examined from the 72 cm- long core. Dinocyst concentrations varied over several orders of magnitude, however, ranging from 434 cysts per ml in the sample from 52 cm to 46,264 cysts per ml only 12 cm deeper in the core (Fig. 3.3). Pollen analysis and varve chronology identified the intervals of peak dinocyst concentration at times of anthropogenic impact and cultural eutrophication during the 14th century and mid 19th to mid 20th century (see Chapter 4). The lowest cyst concentrations (<2000 cysts per ml), from 52 through 42 cm in the core, span the 16th through mid 19th century.

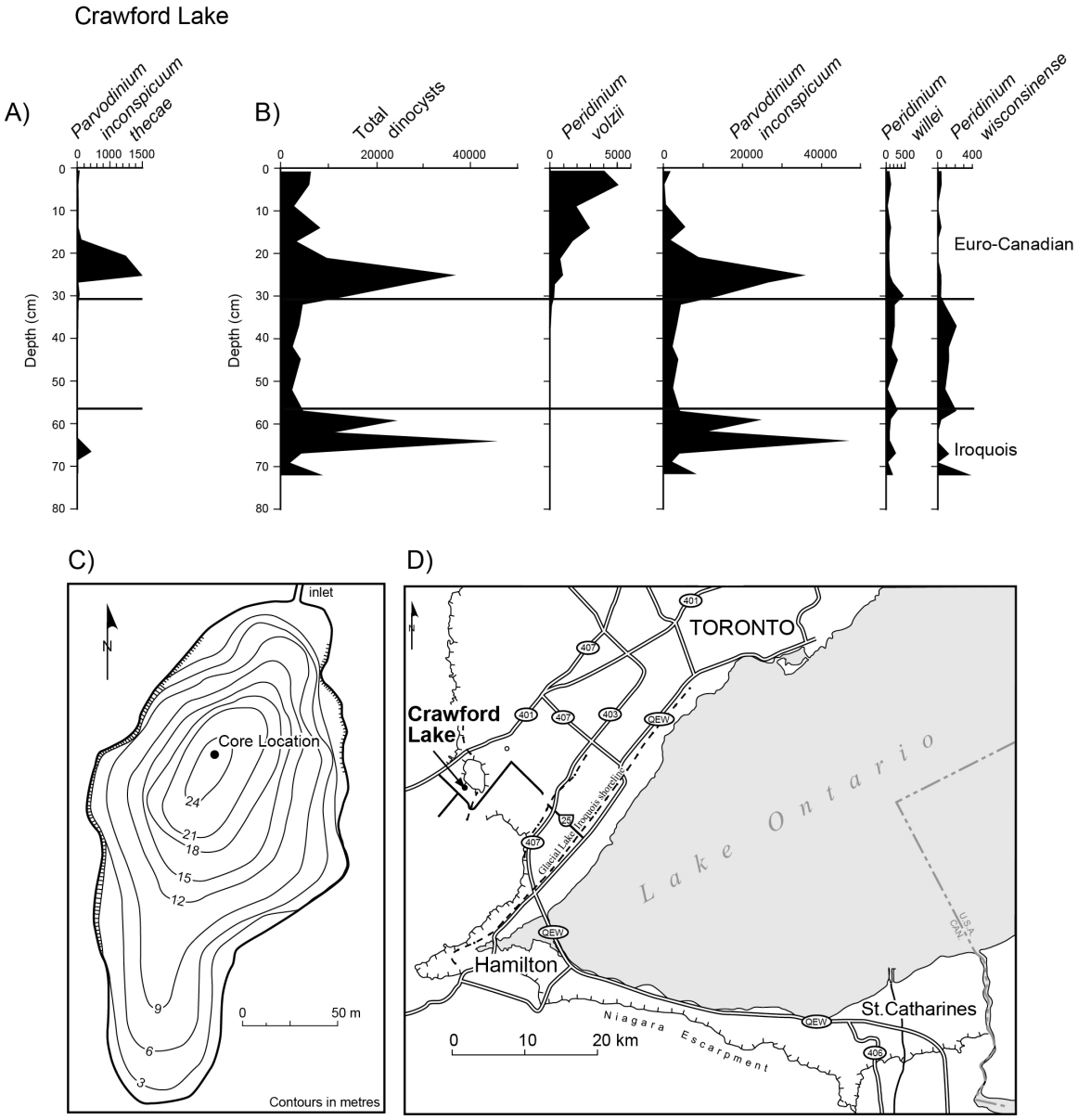

Figure 3.3 Downcore distribution of (A) thecae and (B) cysts in a freeze core collected from (C) the deepest part of (D) Crawford Lake, Ontario, Canada on January 25, 2011. The highest abundance of cysts, from 64 through 59 cm and from 30 through 21 cm is mainly comprised of tiny cysts assigned to *Parvodinium inconspicuum,* whose distinct thecae were also preserved in palynological preparations from 67 cm and in the upper 30 cm of the core, particularly around 21 cm.

Four distinct morphotypes of cysts were identified. Cysts of *Peridinium wisconsinense* Eddy and *Peridinium willei* Huitfeldt-Kaas (Plate 3.1, A-D) are present in low abundances throughout the core, but only make up a significant part of the assemblage below 69 cm and between 57 and 37 cm. The affinity of these cysts has been definitively demonstrated through germinations of both cyst species from small

lakes in sourthern Ontario (McCarthy et al., 2011). Single-cell LSU rDNA analysis on an excysted cell of *P. willei* from Honey Harbour, Ontario (McCarthy et al., 2011) and more recently on cysts of *P. wisconsinense* from Plastic Lake, Ontario (Mertens, personal commun., 2011) have also confirmed the affinity of these cysts. These dinoflagellates are common in lakes from eastern North America (Carty, 2003; McCarthy et al., 2011), where the distinctive morphology of *P. wisconsinense* allows them to be identified in phycological surveys (Nicholls et al., 1992; McCarthy et al., 2011) and where cysts identified informally as "large *Peridinium*" are assumed to be *P. willei* (Sherman, personal commun., 2011). Their cysts have been reported from Holocene lacustrine sediments from Minnesota (Norris and McAndrews, 1970) and southern Ontario (Burden et al., 1986; Zippi et al., 1990, 1991; McCarthy et al., 2011).

Cysts ranging in size from ~38-45 µm by ~42-50 µm initially counted as "small *P. willei*" were reassigned to *Peridinium volzii* Lemmerman (Plate 3.1, E-H) when ovoid and slightly dorso-ventrally flattened thecae with a considerably smaller 1'-plate were observed in the samples from 21 and 25 cm in the core- including specimens of cysts forming within the theca (Plate 3.2, A-D). The cysts are identical to those illustrated by Olrik (1992) and identified as *Peridinium volzii* in their Figs. 14-17. Following, Hansen and Flaim (2007) in distinguishing *P. volzii* and *P. willei* on these grounds, rather than considering them to be conspecific, and *P. volzii* a junior synonym of *P. willei,* as was suggested by Popovsky and Pfiester (1990). The abundance of this cyst in sediments deposited since Euro-Canadian settlement is consistent with the observations of Carty (2003) and Carty and Fazio (1997) that it is common in nutrient-enriched water in North America and Europe. The restriction of these cysts assigned to the upper 32 cm of the core suggests that they were inadvertently introduced when the lake was stocked with sport fish ca. AD 1880 (Crawford Lake Conservation Area, 2011). The association of *P. volzii* with slightly basic pH environments by Olrik (1992) is also consistent with its abundance in recent sediments from Crawford Lake, in a small, deep limestone basin (Krueger et al., 2011), although Carty (2003) and Carty and Fazio (1997) considered this species to be tolerant of a wide range of temperatures and pH, as did Hansen and Flaim (2007).

By far the most abundant cyst in most samples is small (~16-20 µm in diameter), spherical to slightly ovoid in shape, lacking ornamentation, and sometimes with a barely visible sulcal indentation (Plate 3.1, I-L). Its dinoflagellate affinity is only evident in the presence of golden-brown plastids and its double wall of dinosporin. Concentrations of

these tiny cysts exceed 5000 per ml in the samples from 72 cm, from 64 through 59 cm, from 30 through 21 cm, and from 14 cm, and are largely responsible for the large peaks in dinocyst concentration, comprising over 99% of the assemblage in the samples from 64 through 59 cm, for instance. The presence of small thecae with a prominent cingulum and sulcal ridge and a tiny apical horn and often with scattered antapical spines (Plate 3.2, E-G) in several samples from 67 cm and 25 through 22 cm of the core, peaking at ~1475 thecae per ml at 21 cm (Fig. 3.3 A), allows for confidently assigning the tiny, smooth cysts found throughout the core, but most abundantly in these samples, to *Parvodinium inconspicuum* (Lemmermann) Carty. Hansen and Flaim (2007) describe these thecae as small (length: 15-25 µm, width: 15-22 µm) with an ovoid to conical episome that is slightly larger than the hyposome, which is somewhat angular, and a plate formula: Po, x, 4', 2a, 7'', 5''', 2''''. Another observation supporting the assignment of these tiny, unornamented cysts to *P. inconspicuum* was of spongy-walled hypnozygote cysts apparently undergoing meiosis (Plate 3.2, H). These exhibit the "peanut-shaped" morphology reported by Pfeister et al. (1984).

The cysts assigned to this taxon are very different from the dark brown pyriform to ovoid cysts that Chu et al.(2008) attributed to *P. inconspicuum* on the basis of gross morphological similarity (essentially the small size) with the theca of this species. It was not, in fact, present in the water column of the lake they studied, although other thecate dinoflagellates were, notably *Peridinium volzii* Lemmermann and *Ceratium furcoides* (Levander) Langhans. The contention is that the dark brown pyriform cysts that make up the microlaminations in Lake Xiaolongwan, China (Chu et al., 2008) are more similar to cysts identified by Tardio et al. (2006) in Lake Nero, Italy. These could be cysts of *P. volzii*, based on descriptions and photographs, although comparison of slides with the cysts that was quite confidently attributed to this taxon would have to be done in order to assess this possibility, and careful culturing and DNA studies should be undertaken. It is also possible that they are cysts of other small species of *Peridinium* (or *Parvodinium*, following Carty, 2008), e.g. *Parvodinium umbomatum*.

3.4 PALEOECOLOGICAL IMPLICATIONS

The cysts of freshwater dinoflagellates have the potential to be useful paleolimnological proxies, particularly in sediments not conducive to the preservation of siliceous or calcareous microfossils, but several drawbacks exist: 1) it is not known how many

species of freshwater dinoflagellates produce fossilizable resting cysts, although Mertens et al. (2012) report that resting cysts have been described for 84 of the ~350 described species of freshwater dinoflagellates; 2) theca-cyst relationships remain poorly understood; and the identification of thecae to species is not without controversy; and 3) little is known about the ecological and taphonomic factors that control the biogeographic and stratigraphic distribution of dinocysts in lacustrine sediments (McCarthy et al., 2011; McCarthy and Krueger, in press; Mertens et al., 2012).

The exceptional preservation in this Holocene lagerstatte allowed for the identification of four species of Peridinales cysts that appear to vary in absolute and relative abundance through time in response to two documented phases of cultural eutrophication. The abundance of cysts attributed to *Parvodinium inconspicuum* (Lemmermann) Carty and to *Peridinium volzii* Lemmermann in sediments impacted by humans is consistent with their ecology and biogeography, but their cysts have not previously been reported in sediments from North American lakes. The exceptional preservation in the meromictic waters of Crawford Lake that allowed thecae to be preserved together with very high concentrations of dinocysts, at least some of which remained viable for centuries, may provide a taphonomic explanation for their occurrence. Enhanced preservation in quiet, low oxygen environments has been identified as a contributing factor in other studies (McCarthy et al., 2011). The lack of reports of cysts of these taxa in sedimentary records of lakes could also reflect the failure of palynologists to notice these small, relatively non-descript cysts on their slides and attribute them to dinoflagellates. Evidence that this provides at least a partial explanation comes from the identification of cysts of *Parvodinium inconspicuum* during a rapid re-examination of slides from core SV5-C from Honey Harbour, previously analysed by both F. McCarthy and K. Mertens (McCarthy et al., 2011). Further work is required to determine how widespread these cyst taxa are in other lakes lacking such exceptional preservation and to confirm the identification of resting cysts of *Parvodinium inconspicuum* and *Peridinium volzii.*

3.5 CONCLUSIONS

The preservation of theca in varved sediments deposited in Crawford Lake at times of cultural eutrophication, together with the inadvertent culturing of dinoflagellates from the top sample down to 29 cm (~A.D. 1845-1860) during palynological processing, allowed

cysts to be confidently assigned to two species of dinoflagellates not previously described from the Great Lakes region of North America: *Parvodinium inconspicuum* (Lemmermann) Carty and *Peridinium volzii* Lemmermann. These may be present in higher concentrations in this lagerstatte than would be expected in non-meromictic lakes from this region, but we suggest that the major reason that these cysts have not previously been reported is the failure of palynologists to recognise their affinity.

3.6 SYSTEMATIC TAXONOMY

SUPERKINGDOM Eukaryota
KINGDOM Chromalveolata
SUPERPHYLUM Alveolata Cavalier-Smith 1991
PHYLUM Dinoflagellata Butschli 1885
CLASS Dinophyceae Pascher 1914
ORDER Peridiniales Haeckel 1894
FAMILY Peridiniaceae Ehrenberg 1831
GENUS *Parvodinium* Carty 2008
SPECIES *P. inconspicuum* (Lemmerman 1899) Carty 2008
GENUS *Peridinium* Ehrenberg 1832
SPECIES *P. volzii* Lemmerman 1904
P. willei Huitfeld-Kaas 1900
P. wisconsinense Eddy 1930

3.7 TAXONOMIC DESCRIPTIONS

Cysts of *Peridinium wisconsinense* Eddy (Plate 3.1, A & B) easily identified by the presence of a rounded, sometimes bifurcated apical horn and a single, sharply pointed antapical horn. The cysts are cavate and proximate, with a smooth, transparent, slightly ellipsoidal inner layer, and an ornamented outer layer appressed to the inner layer in the equatorial region. The archeopyle, when observable, encloses an operculum, and is composed of the apical plates 2′, 3′ and 4′ and part of the first apical plate (1′). The length from apical to antapical horn of the outer layer of the cysts from varved sediments from Crawford Lake is 63 µm (stdev=4 µm) and the width is 50 µm (stdev=3 µm).

Cysts of *Peridinium willei* Huitfeldt-Kaas (Plate 3.1, C & D) are cavate and proximate, with both layers closely appressed, although they sometimes can detach as noted in

McCarthy et al. (2011). The inner layer is transparent, smooth and ellipsoidal, and the outer layer is slightly invaginated in the sulcal area forming two distinct shoulders. Where sutures can be discerned, the archeopyle can be seen to be transapical (McCarthy et al., 2011). Cysts attributed to this genus in the varved sediments from Crawford Lake range in size from 48 to 58 µm by 49 to 52 µm (stdev=2 µm).

Cysts of *Peridinium volzii* Lemmerman (Plate 3.1, E-H) are cavate and proximate, but lacking ornamentation. They are smaller (~38-45 µm by ~42-50 µm) than cysts of *P. willei*, which they resemble, but they have less pronounced shoulders and lack an apical flange

Cysts of *Parvodinium inconspicuum* (Lemmermann) Carty (Plate 3.1, I-L) are tiny ~ 15-22 µm, spherical double-walled cysts lacking ornamentation and sometimes with a barely visible sulcul indentation. The red nuclei within the cyst indicate viable cell contents.

Thecae of *Peridinium volzii* Lemmerman (Plate 3.2, A-D) are similar to *P. willei* but smaller in size, and ovoid and slightly dorso-ventrally flattened thecae with a considerably smaller 1'-plate. Their tabulation is illustrated in Fig. 3.1 (C &D). Thecae of *P. volzii* found in our samples are usually broken along the posterior edge of the cingulum, similar to the observations of Boltovskoy (1984) for *P. willei*. *P. volzii* have been found in lakes from North America and Europe (Hansen and Flaim, 2007; Carty, 2003; Carty and Fazio, 1997; Olrik, 1992) across a wide range of temperatures and pH. They are most abundant in eutrophic environments (Olrik, 1992).

Thecae of *Parvodinium inconspicuum* (Lemmermann) Carty (Plate 3.2, E-G) are approximately 20-25 µm by 20-30 µm in size with a pronounced apical horn and scattered antapical spines, although the number and size of these spines is variable. Hansen and Flaim (2007) point out that Lemmermann (1910) distinguished *P. inconspicuum* from the very similar *P. umbonatum* Stein by the presence of antapical spines in *P. inconspicuum*, but they note that this appears to be a very variable character of this species. As the name implies, thecae of *P. inconspicuum* are inconspicuous due to their small size- about half the size of other dinoflagellate species (Koryak, 1978; Shen and Zhang, 1990; and Carty, 2003). *P. inconspicuum* is found in

freshwater lakes, ponds and marshes in the Americas and Europe and is most abundant with a low pH (Perez et al., 1994; Koryak, 1978; Moiseenko, 2005; Havens and De Costa, 1987).

Hypnozygotes of *Parvodinium inconspicuum* (Lemmermann) Carty (Plate 3.2, H) are enlarged spherical unarmored spongy-walled cells with the zygotic nuclei centrally located and ~25-30 µm in diameter. The hypnozygote is the product of sexual reproduction two armoured motile gametes. After the fusion of each gametes protoplast a zygote forms and develops its own theca. The armoured zygote eventually settles to the substrate where it sheds its theca and emerges as a hypnozygote ready to undergo meiotic division.

3.8 PHOTOPLATES

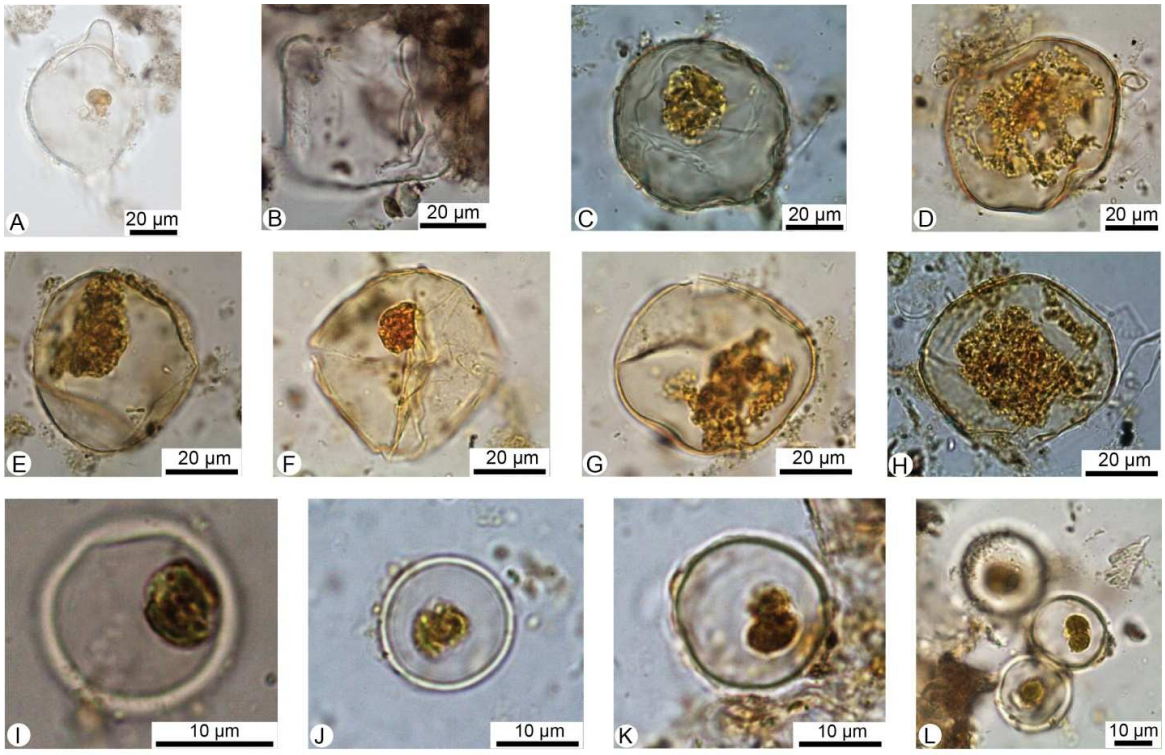

Plate 1.

Plate 1

A,B Cavate, proximate cysts of *Peridinium wisconsinense*, easily identified from their rounded, sometimes bifurcated apical horn and a single, sharply pointed antapical horn, measuring ~62 µm from horn to horn.
C,D Cavate, proximate cysts of *Peridinium willei*, with the outer layer slightly invaginated in the sulcal area to form two distinct shoulders that together with the larger size (~50 by 60 µm) distinguish it from cysts of *P. volzii.*
E-H *Peridinium volzii* small (~30 by 40 µm), unornamented cavate, proximate cysts with distinctive red bodies (F).
I-L Tiny (~16-20 µm in diameter) unornamented spherical cysts of *Parvodinium inconspicuum*, commonly containing red bodies.

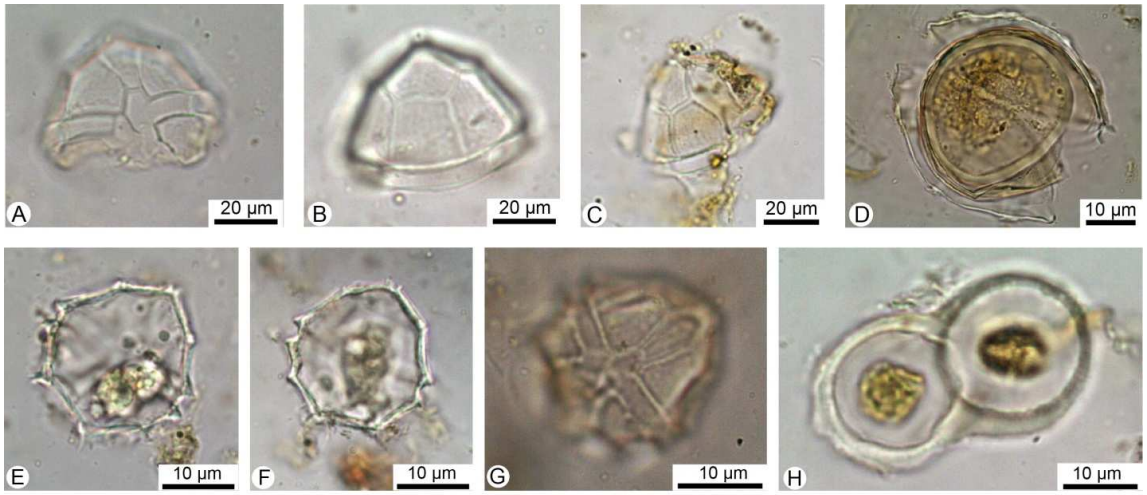

Plate 2.

Plate 2

A-C Ventral and dorsal views of an ovoid and slightly dorso-ventrally flattened theca of *Peridinium volzii* Lemmerman, similar to *P. willei* but smaller in size (~38-45 µm by ~52-60 µm), and with a considerably smaller, 5-sided 1'-plate.
D *Peridinium volzii* cyst emering from its theca found in a cultured palynological preparation.
E-F Ventral and dorsal views of a small (~20-25 µm by 20-30 µm) theca of *Parvodinium inconspicuum* (Lemmermann) Carty, showing the pronounced apical horn and scattered antapical spines. These are preserved in palynological preparations of varved sediments from Crawford Lake.
G Ventral perspective of a *Parvodinium inconspicuum* theca with clearly identifiabletabulation.
H Hypnozygotes of *Parvodinium inconspicuum* just completing a meiotic division with spongy walls and vivid orange body centrally located.

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Chapter 4. The cultural eutrophication of Crawford Lake – insights from dinoflagellates

4.1 Introduction

Cultural eutrophication, the enrichment of aquatic environments with excess nutrients due to human influences, is the most common water quality problem around the world (Gloterman et al., 1975; Schindler, 1977). Human activity (land clearance, agriculture, urbanization, and industrialization) has had detrimental affects on numerous ecosystems and disturbed many natural chemical cycles, increasing soil erosion and the release of nutrients into groundwater and surface runoff from waste and emissions (Ekdahl, 2004; Klug and Cottingham, 2001).

The input of limiting nutrients, especially during times when nutrient levels are usually low (i.e. late summer), results in excessive algal and plant growth, leading to a depletion of dissolved oxygen levels due to increased biochemical oxygen demand as decay occurs, as well as a decrease in light penetration. The crash in fisheries around the world caused by algal blooms and the decline in the quality of our freshwater reservoirs has led to the identification and impact of cultural eutrophication on ecosystems. Long term trends have to be studied in order to understand present conditions and prevent future eutrophication of our waterways by implementing sustainable management measures. The response of critical freshwater resources to environmental perturbations (both natural and anthropogenic) can be assessed using time series of environmental data recorded by microfossils preserved in lake sediments in a way that synoptic measurements of water quality cannot.

Although dinoflagellates are an important component of the summer phytoplankton in lakes, their cysts have seldom been used as paleolimnological proxies. Little work has been done on the ecology of freshwater dinoflagellates, and even less on the biogeographic distribution and taphonomy of their resting cysts, but over the last few decades a number of palynological investigations in the southeastern Great Lakes region of Ontario, Canada have identified cysts attributed to various species of *Peridinium* and attempted to relate their stratigraphic and biogeographic distribution to environmental conditions in the watershed (Fig. 4.1). This paper investigates the dinoflagellates response to two known intervals of human settlement (Iroquois in the 13th -15th centuries and Euro-Canadian since the early 19th century) in the Crawford Lake

watershed, using varve counting to establish a chronology and pollen to illustrate land use practices.

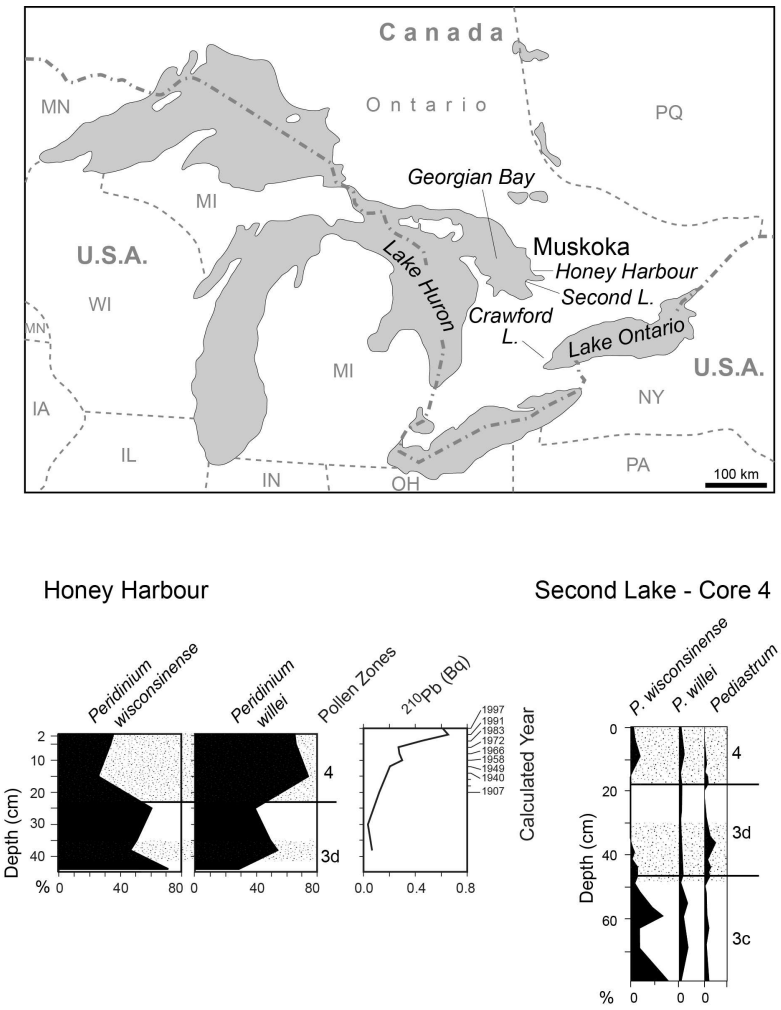

Fig. 4.1 Location of sites in the southeastern Great Lakes region where dinocyst distribution has been related to ecological parameters, notably anthropogenic impact (Second Lake- Burden et al., 1986; Honey Harbour- McCarthy et al., 2011; Crawford Lake- this study). Stippling identifies core horizons with evidence of Wendat (Huron) and Euro-Canadian impact on the watersheds of Second Lake (now in a Provincial Park) and Honey Harbour.

4.2 Crawford Lake - geographical and geologic setting

Crawford Lake (43°28'N, 79°57'W) is situated near t he edge of the Niagara Escarpment ~50 km west of Toronto and 278 m above sea level (Figure 4.2). The climate around Crawford Lake is humid continental with warm summers and cold winters (Bryson and Hare, 1974; Rowe, 1972). The small (2.4 ha) lake occupies a deep dolomite (Silurian Lockport Fm.) basin, thought to have been excavated by hydraulic mining (McAndrews

and Boyko-Diakonow, 1989). Water enters the lake mostly by seepage from a catchment of ca 80 km^2 (Yu et al., 1997).

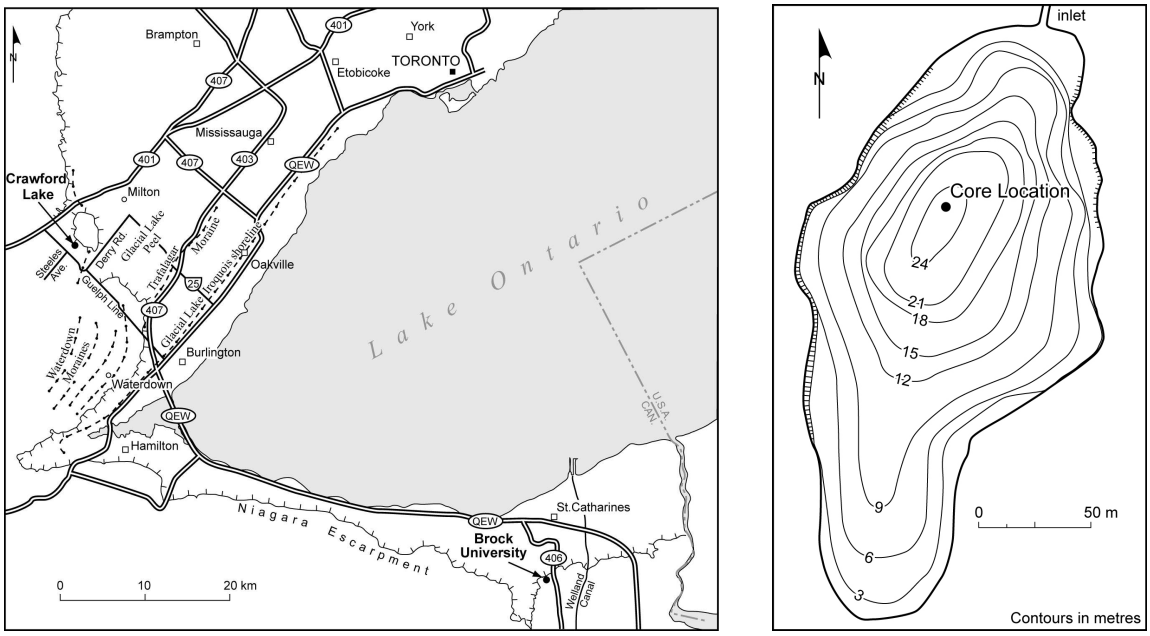

Fig. 4.2 The core was taken using a frigid finger nail sampler from the deepest part of Crawford Lake, in >24 m water. The small, deep lake on the edge of the Niagara Escarpment is meromictic, allowing varves to accumulate.

The lake does not fully overturn due to the relatively small surface area (~250 x 150 m) and great depth (up to 24 m of water overlies ~4.5 m of post glacial sediments in the deepest part of the basin) (Turton & McAndrews, 2006: Yu, 2003). Wind protection is further enhanced by the 6 m high dolomite cliffs and old growth forest dominated by eastern white cedar (*Thuja occidentalis*) that surround the lake in this region near the boundary between the deciduoius and Great Lakes mixed forest of Rowe (1972) (Table 4.1). Meromictic conditions result, with water below 15 m lacking oxygen and having a constant temperature of 5-6 ºC (McAndrews and Turton, 2010). Meromixis allowed annually laminated sediments to accumulate over the past ca 2000 years (Yu, 2003). The laminations form in couplets of a white calcite-rich layer (deposited in spring/ summer) and a dark organic-rich layer (deposited in late fall) (Dickman, 1979). During the fall, wind mixing of the mixolimnion results in the introduction of oxygen into the chemocline (below 15 m) resulting in a mass mortality of photosynthetic bacteria. The bacteria release sulphur as they are sinking and decomposing that reacts with the ferrous ions in the chemocline to produce black ferrous sulphides and pyrite resulting in

dark organic rich lamina deposited each fall. In late spring and summer when water temperatures are at their highest, calcite starts to precipitate out, depositing a white calcium rich lamina (Dickman, 1979). The anoxic bottom waters are unable to support benthic organisms thus the lamina deposited each year are well preserved due to a lack of bioturbation (Fig 4.3).

Table 4.1 Common tree taxa in the Crawford Lake catchment near the boundary between Deciduous Forest and the Great Lakes – St. Lawrence Mixed Forest of Rowe (1972). Annual precipitation is 973 mm, mean summer temperature 20.6ºC, and mean winter temperature -6ºC (Environment Canada, 2011).

sugar maple	*Acer saccharum*
white birch	*Betula papyrifera*
beech	*Fagus grandifolia*
white ash	*Fraxinus americana*
ironwood	*Ostrya virginiana*
white pine	*Pinus strobus*
red oak	*Quercus rubra*
eastern white cedar	*Thuja occidentalis*
basswood	*Tilia americana*
hemlock	*Tsuga canadensis*
white elm	*Ulmus americana*

Varve counting allows for a very precise chronology, although some discrepancies have been noted for the Post Iroquoian period where ~10% of the varves are missing when compared to AMS dates (Ekdahl et al., 2004 & 2007). Chan et al., (submitted) found that benthic anoxia might have subsided episodically towards the end of the Post Iroquoian Zone based on ostracodes analysis that are excellent paleo proxies for dissolved oxygen in freshwater ecosystems.

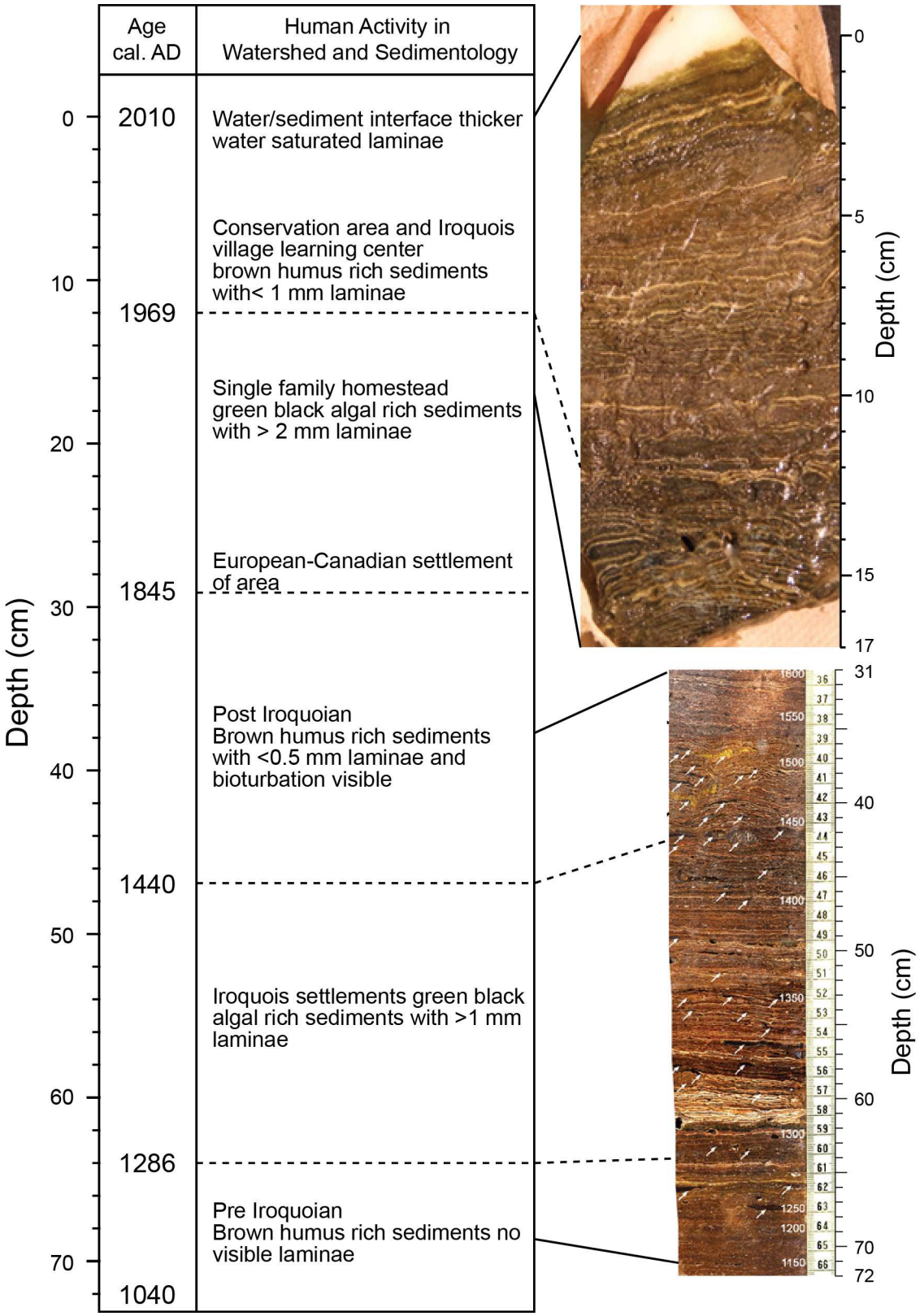

Fig. 4.3.a Photograph of the upper 12 cm of the freeze core collected on January 25, 2011 showing light and dark couplets, and interpreted varve chronology across the Iroquois interval from a core collected in 2011 in McAndrews and Turton (2007).

Fig. 4.3.b Sampling part of the freeze core for palynological analysis (clockwise: K. Noseworthy, *The Weather Network*, Calvin Chan and Charlie Turton, University of Toronto, Andrea Krueger and Francine McCarthy, Brock University).

4.3 Methods

A 72 cm long freeze core (Fig.4.3) was recovered in January of 2011, from the deepest point of Crawford Lake (24 m) using a frigid fingernail, a hollow aluminum wedge filled with ethanol and dry ice slurry. Abbreviated film footage of coring from the ice surface can be viewed at: *http://www.theweathernetwork.com/news/storm_watch_stories3&stormfile=ontario_lake_holds_climate_c_040411*. All cores were photographed before planing and scraping the outer surface to reveal the undisturbed frozen varves. The exceptionally well preserved annual laminae consist of a couplet consisting of a white layer that is very rich in calcite and forms during the summer and a dark layer in each couplet and is rich in organic material and forms during the fall (Dickman, 1979). Sediment samples were taken every 5 centimetres to determine intervals of human habitation. Higher resolution samples were taken every 2 cm across periods of anthropogenic impact, with each sample therefore representing 4-5 years of accumulation. The samples were processed using a

weak (10%) HCl solution spiked with a tablet containing 10,850 +/- 200 spores of *Lycopodium clavatum* (batch # 006720). The samples were then sieved using distilled water and a 15 µm Nitex sieve and mounted to slides using glycerine jelly.

The strew slides were examined using a Leica DMLB light microscope at 400X magnification and photographed using oil immersion at 1000X magnification using a Leica EC3 Digital Imaging Camera.

4.4 Results

Four distinct dinocyst morphotypes were identified in the sediments from Crawford Lake (Photo Plate 4.1), 3 species attributed to the genus *Peridinium* and one newly reassigned to the genus *Parvodinium* Carty (Chapter 3, McCarthy and Krueger, in press). Two of these cysts have been related to the species *Peridinium wisconsinense* Eddy and *Peridinium willei* Huitfeld-Kaas in previous studies (Wall and Dale, 1968; McCarthy et al., 2011). Two other common cyst species in varved sediments from Crawford Lake have been assigned to *Parvodinium inconspicuum* (Lemmermann) Carty and *Perdinium volzii* Lemmermann based on culturing observations as well as the exceptional preservation of thecae of *P. inconspicuum* in palynological preparations from a few levels in the core, typically associated with cyst concentrations several orders of magnitude higher than the average (Chapter 3, McCarthy and Krueger, in press)

Cyst concentrations range from 434 to 46,264 cysts per ml during the ~ 900 years recorded in the core, with peak concentrations associated with abundant non-arboreal pollen (NAP) (Fig. 4.4 &4.5 a). *Helianthus* (sunflower), Gramineae/Poaceae (including Zea), and *Portulaca* (purslane) are found in consistent high abundances from the base of the core through ~A.D. 1400 – 1486. Ambrosia (ragweed), Gramineae/Poaceae (including Zea), Chenopodiaceae, and *Rumex* (dock and sorrel) occurs in very high abundances from ~A.D. 1820 to the surface (the ragweed/ *Ambrosia* rise), based on varve counts and historical documents (Fig. 4.5 b). The dinocyst assemblage in the Iroquois sediments consists of *P. inconspicuum, P. willei* and *P. wisconsinense*, with abundances of *P. inconspicuum* reaching a peak of ~46,000 cysts/ml at ~A.D. 1300 (just below the peak in NAP, Fig 4.5 a). Low concentrations of these taxa persist from ~A.D. 1486 to ~A.D. 1820, when concentrations increase sharply again, coinciding with a colour change- the greenish colour reflecting the large quantities of algal bodies being incorporated into the sediments and/or increasingly reducing

conditions. *Peridinium volzii* makes its first appearance in the sediments from Crawford Lake at ~ A.D. 1880.

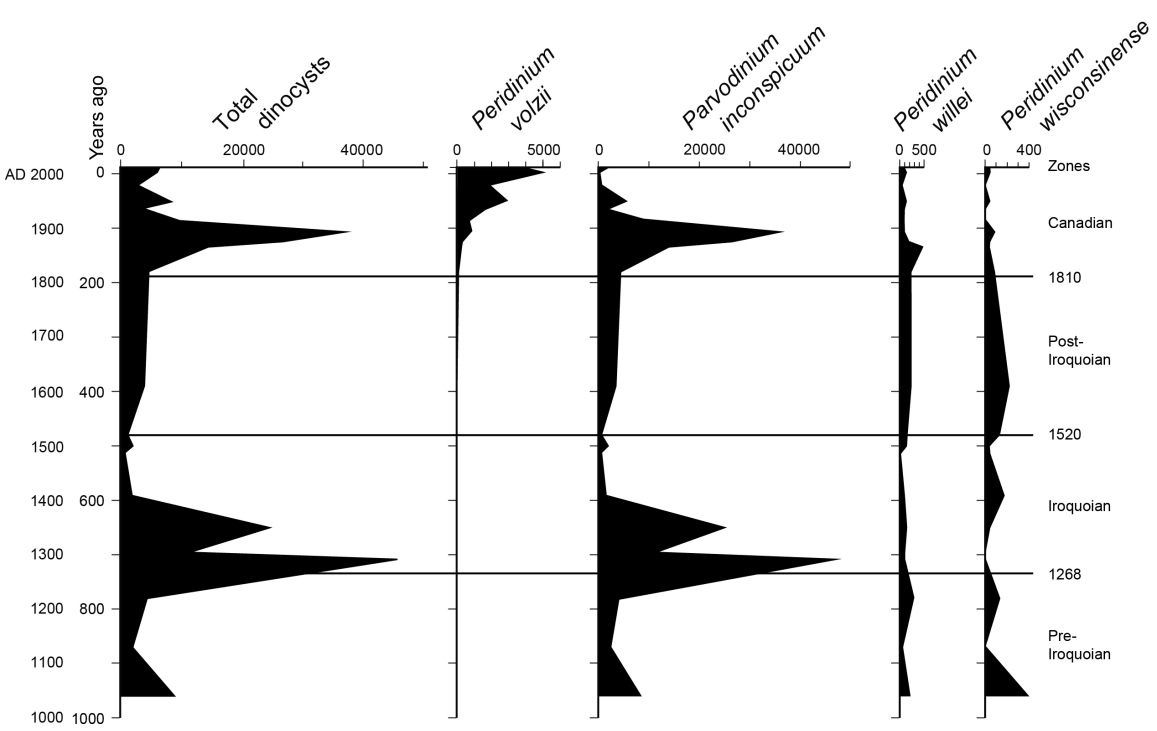

Fig. 4.4 Dinocyst data (cysts/ml) plotted against age, interpreted from varve counting, following the methodology of Ekdahl et al. (2004 & 2007). Dramatic increases in cyst abundance in the 13th -14th centuries and in the late 19th century, primarily comprised of *Peridinium inconspicuum*, reflect an increase in nutrient flux and decrease in pH associated with Iroquois and Euro-Canadian impact in the watershed.

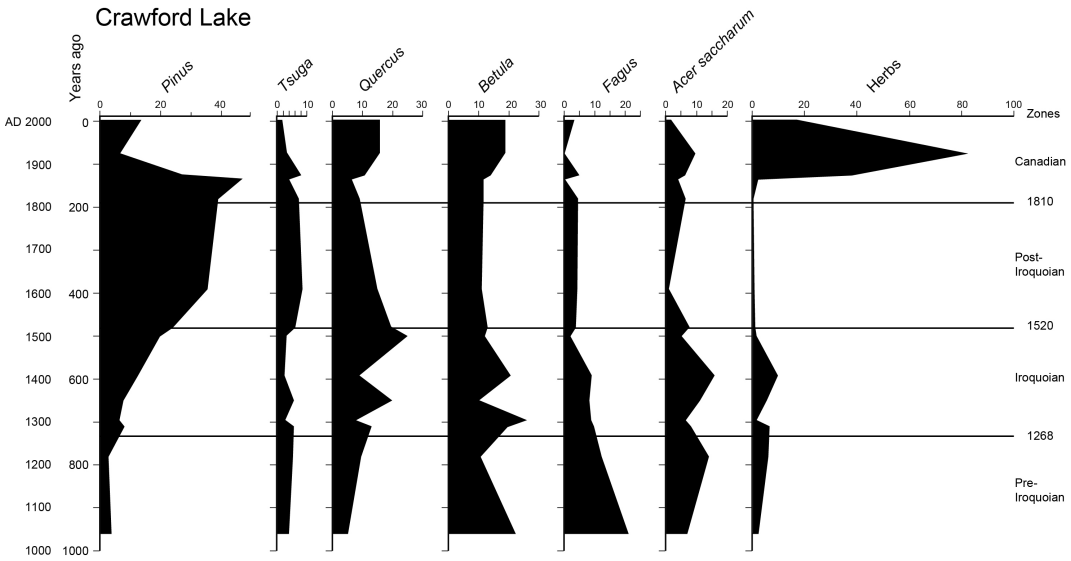

Fig.4.5 a. The relative abundance of selected pollen data plotted against age, interpreted from varve counting, following the methodology of Ekdahl et al. (2004 & 2007). Peaks in herb pollen record land clearance for settlement and agriculture during the Iroquois interval and deforestation for the lumber mill during the late 19th C and subsequent agriculture until AD 1969. Units in percent of total counted dinocysts.

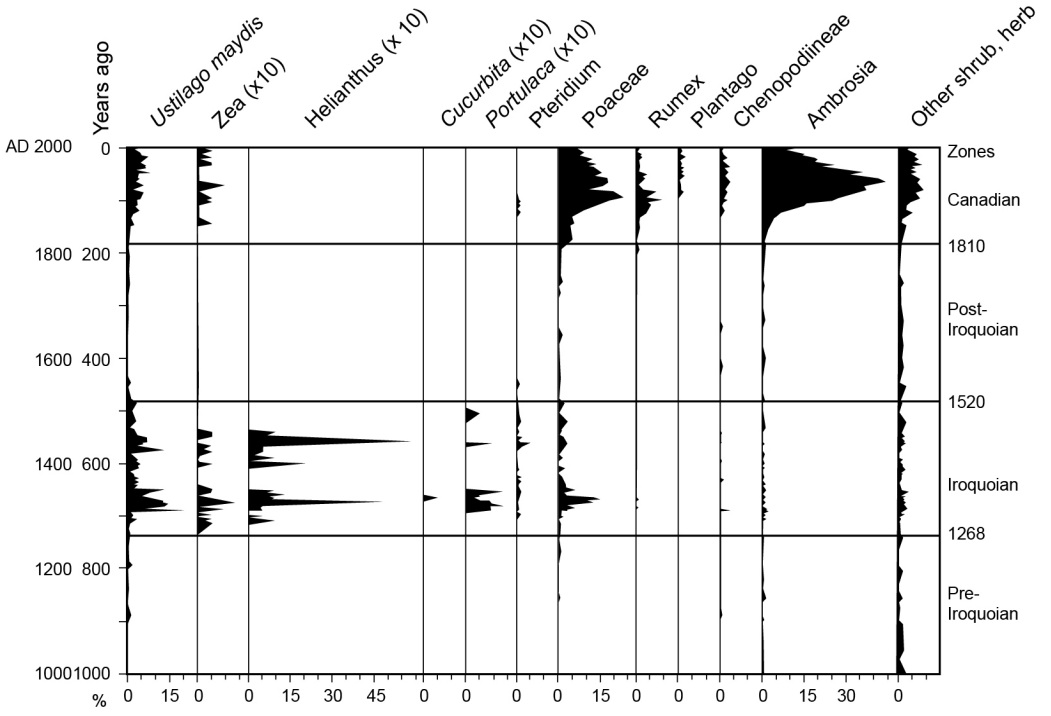

Fig. 4.5 b. The relative abundance of selected herb pollen data plotted against age, interpreted from varve counting, following the methodology of Ekdahl et al. (2004 & 2007).

4.5 Discussion

Native Iroquois people lived in agricultural villages during the 13th to 15th centuries within 3 km of the lake with a small site 150 m from the lake intermittently occupied by ca 200-300 people during the 1400's (Finlayson 1998; Byrne and Finlayson, 1998; Dodd et al., 1990). The presence of corn (*Zea)*, Purslane (*Portulaca)*, and Sunflower (*Helianthus)* pollen in the samples analyzed from ~A.D. 1400 to 1486 is indicative of Iroquois agricultural practices as found in previous studies (McAndrews and Boyko-Diakonow, 1989; Boyko-Diakonow 1979; Boyko, 1973). Spores of corn smut (*Ustilago maydis)* have also been found at this time period (McAndrews and Turton, 2010) a common parasite of corn which was a staple crop for native Iroquois. The abundance of these indicators from ~AD 1400-1486, associated with a sharp decline in dinocyst abundance (and presumed nutrient flux) suggests runoff of pollen into the lake from an abandoned village during the late 15th C.

The dominance of *P. inconspicuum* in the cyst assemblage from the Iroquois interval is consistent with the tolerance of this species to eutrophic conditions (Koryak, 1978, Moosienko, 2005). *P inconspicuum* (Lemmermann) Carty has been found in freshwater environments in the North America (Perez et al., 1994; Koryak, 1978) and Europe (Fott et al., 1999; Moiseenko, 2005; Holopainen, 1991) and is found to be abundant in waters with a pH as low as ~4.5 (Perez et al., 1994). Perez et al. (1994) found that when pH is low ~4.5 addition of limiting nutrients (P and N) had no discernible affects on phytoplankton growth, but when pH was increased to above pH 6 phytoplankton composition changed greatly in response to the addition of nitrogen and phosphorous. Koryak (1978) observed that in minerally acid waters of the Upper Ohio River basin *P. inconspicuum* dominated the phytoplankton communities. The affinity that *P.inconspicuum* has for eutrophic water regimes has also been observed by Moiseenko (2005), Fott et al. (1999) and Umana (2001) whether it be naturally acidic (crater lakes) or anthropogenically influenced.

The dramatic increase in dinocyst concentrations (an order of magnitude) together with the change in dinocyst assemblages suggest that the Iroquois agricultural practices and village refuse affected dinoflagellate communities through increased nutrient input leading to enhanced productivity. This was augmented by Canada geese that grazed on the Iroquois fields in the fall, and then roosted at Crawford Lake for the night depositing dung pellets rich in pollen and spores from cultivated crops (McAndrews and Turton, 2007). In agreement with previous paleolimnological studies done at

Crawford Lake at this time, the diatom assemblage consisting of *Cyclotella michiganiana* and *Cyclotella bodanica* (meso-oligotrophic species) was replaced by *Synedra nana*, *Fragilaria crotonensis* and *Asterionella Formosa,* all species that thrive at higher concentrations of nutrients especially phosphorus (Ekdahl et al., 2004 & 2007) and rotifer loricas (microscopic herbivores that feed on single celled algae and bacteria) became an abundant component of the ecosystem at this time as well, driven by the increased abundance of their food supply (Turton and McAndrews, 2006).

The abandonment of the Iroquoian village sites around Crawford Lake caused dinocyst concentrations to decline quite substantially reflecting a decreased input of nutrients into the catchment area. *P. wisconsinense* abundance increases during this time period and indicates improved water quality and a decrease in human effluent as this species has been associated with oligotrophic to mesotrophic conditions in other studies in southern Ontario (McCarthy and Krueger, in press; McCarthy et al., 2011). *P. wisconsinense* has only been reported from mesotrophic (Meyer, 1969) to oligotrophic (Canion and Ochs, 2005) ponds and lakes in North America (Wall & Dale, 1968, Norris & McAndrews, 1970; Carty, 2003; McCarthy et al., 2011), and its cysts are restricted to surface sediments in lakes with low total phosphorus concentrations (TP<5) (Zippi et al., 1991), preferring neutral pH (Cottingham et al., 1998 and McCarthy et al., 2011). Ekdahl et al. (2004 & 2007) noted that there was a reduction in diatom productivity, but the assemblage dynamics stayed in an altered state and did not reflect the same changes that the geochemical data indicated. The abundance of two species of rotifers, *Keratella hiemalis* and *Kellicottia spp.* decreases only slightly during the post-Iroquoian zone (Turton and McAndrews, 2006). Dinocysts thus appear to be more sensitive to the recovery in water quality between the two phases of human settlement.

Canadian farmers acquired land between AD 1822 and 1864 within the catchment area of Crawford Lake (McAndrews and Boyko-Diakonow, 1989) and cleared forests for farming. The Canadian zone starting AD 1841 is marked by a change in colour as well as an increase in ragweed (*Ambrosia)*, grass (Graminae*)* and a decrease in white pine (*Pinus strobus)* due to selective logging. Dinocyst concentrations from approximately 1820 to the mid 1860's, increase slightly due to the human activity around the lake. During this time period the dinocyst assemblage still consisted of *P. inconspicuum, P. willei* and *P. wisconsinense*.

During the 1880's a lumber mill was erected on the south end of the lake (Fig. 4.6) and fish were introduced into the ecosystem that was fishless (Crawford Lake

Conservation Area, 2011). The lumber mill increased nutrient flux entering the lake and increased burial rates leading to the preservation of dinoflagellate thecae in the well preserved sediments. *P. inconspicuum* increases markedly during the time the lumber mill is active and there is a slight increase in *P. willei*, species that tolerate mesotrophic to eutrophic waters, whereas *P. wisconsinense* declines due to the increasing nutrient loading. For the first time in the biostratigraphy of Crawford Lake *Peridinium volzii* appears around this time, most likely introduced into the lake at the same time they stocked the lake with trout and bass for recreational fishing (C. Turton, *pers. commun*., 2011). *P. volzii* have been found in lakes from North America and Europe across a wide range of temperatures and pH, most commonly in eutrophic environments (Hansen and Flaim, 2007; Carty, 2003; Carty and Fazio, 1997; Olrik, 1992). In a core from Sluice Pond, Massachusetts, *P. volzii* covaries with magnetic susceptibility and increased industrial activity and acid rain that bring many airborne toxins from greater distances than runoff and seepage alone.

Fig. 4.6 Photograph taken of the lumber mill located at the south end of Crawford Lake (Crawford Lake Conservation Area, 2011)

At the beginning of the Canadian zone *P. volzii* was only a minor part of the dinocyst assemblage, but there is a change during the 1930's from a *P. inconspicuum*

dominated assemblage to one dominated by *P. volzii.* The lumber mill was abandoned by this time and diatom production was reduced, indicating recovery of the ecosystem during the period of homesteading by the Crawford family (Ekdahl et al., 2004). $CaCO_3$ precipitation increased in Crawford Lake around this time, probably resulting from increased algal production utilizing aqueous CO_2 and raising pH (Ekdahl et al., 2004). This could explain the turnover to *P. volzii* from *P. inconspicuum*, because *P. inconspicuum* prefers acidic environments (although it tolerates a wide range of pH regimes: 3.5-6.5), while *P. volzii* has been found to be most prolific in neutral to basic water bodies (pH 6.5-8), although it too tolerates a wide range of pH (Olrik, 1992).

In 1969, and archeological survey of the north slopes of Crawford Lake was undertaken (Finlayson, 1998; Byrne, 1998; Byrne and Finlayson 1998), and in 1972, the Crawford's house and barn were replaced by a reconstructed Iroquoian village and visitor center, and the area surrounding Crawford Lake became a conservation area, controlled by Conservation Halton. This is recorded by the decrease in non-arboreal (herb) pollen toward the top of the core, and a sharp decline in cysts of *P. inconspicuum*, although cysts attributed to *Peridinium volzii* reach peak abundances on the modern lakebed.

There are differences between the two periods of occupation around Crawford Lake. Dinocyst concentrations were greater during the Iroquoian period than the Euro Canadian period, but the Iroqouis assemblage was almost monospecific. The Iroquois did not till vast amounts of land to plant crops, and had a three tiered planting system where corn held up the beans and shades the squash, leaving very little uncovered soil that could be eroded away. The major nutrient flux (primarily nitrogen) to the lake would have been from Canada Geese defecating in the lake and most likely the cause of eutrophication and the accompanying meromixis (McAndrews and Turton, 2007) as found in other small lakes where waterfowl roost (Manny, 1994). Fecal pellets belonging to *Branta Canadensis* (Canada goose) containg *Helianthus* (Sunflower) pollen and seeds along with Zea maize (corn) pollen restricted to the Iroquois interval indicating that Canada Geese fed in the Iroquois corn fields (McAndrews and Turton, 2007). After feeding on the Iroquioan crops the geese roosted at Crawford Lake defecating pellets that provided nutrients for algal blooms and subsequent eutrophication (McAndrews and Turton, 2007). The Euro-Canadians impacted the ecosystem by introducing large amounts of plant matter into the lake during logging, increasing siltation into the lake due to more extensive deforestation, and introducing sport fish and (inadvertently) *P. volzii*.

Euro-Canadian farming practices involved tilling vast fields twice a year, liberating large quantities of nutrients into the water system and increases aeolian input due to increased erosion of the land, also crops are monospecific planted in discrete rows that leaves large areas of uncovered soil further increasing erosion thus nutrient and sediment load. The use of fertilizers (nitrogen & phosphorus) and pesticides (DDT), together with industrial and automobile emissions (heavy, metals and acid rain) (Ekdahl, 2004; Klug and Cottingham, 2001) could have detrimental effects on the natural biota and water chemistry of the lake and its productivity in the 20th century.

4.6 Conclusions

Downcore variations in dinocyst concentrations indicate two periods of increased cyst preservation that covary with both settlement intervals, prehistoric Iroquois (~A.D. 1286 – 1486) and historic Euro-Canadians (since ~A.D. 1820). The cysts of dinoflagellates are visible in the well preserved sediments found at Crawford Lake. Also times of enhanced sedimentation rates, the beginning of the Iroquioan interval and the Euro-Canadian zone, further depleted dissolved oxygen levels leading to the preservation of the cellulosic thecae of *P. inconspicuum* as well as high numbers of cysts. The absolute abundance of dinocysts increases with nutrient flux associated with both Iroquoian and Euro-Canadian impact, and *Peridinium wisconsinense* is generally replaced by other *Perdinium* species during times of human activity around the lake, consistent with the observation that P. wisconsinense is not well adapted to eutrophic conditions. During the Euro-Canadian zone *Peridinium volzii* makes its first appearance in the dinocyst assemblage and eventually replaces *Parvodinium inconspicuum* as the most dominant species. It is thought *P. volzii* was introduced into the Crawford Lake ecosystem by Euro-Canadian settlers stocking the lake with fish. The dinocyst record also corresponds to that of diatoms, rotifer loricas, fungal spores and geochemical data found to be reliable proxies for cultural eutrophication. There is also a strong correlation with the pollen record that indicates periods of deforestation and nearby agriculture activities in regards to the two periods of occupation in the Crawford Lake catchment area.

4.7 PHOTOPLATES

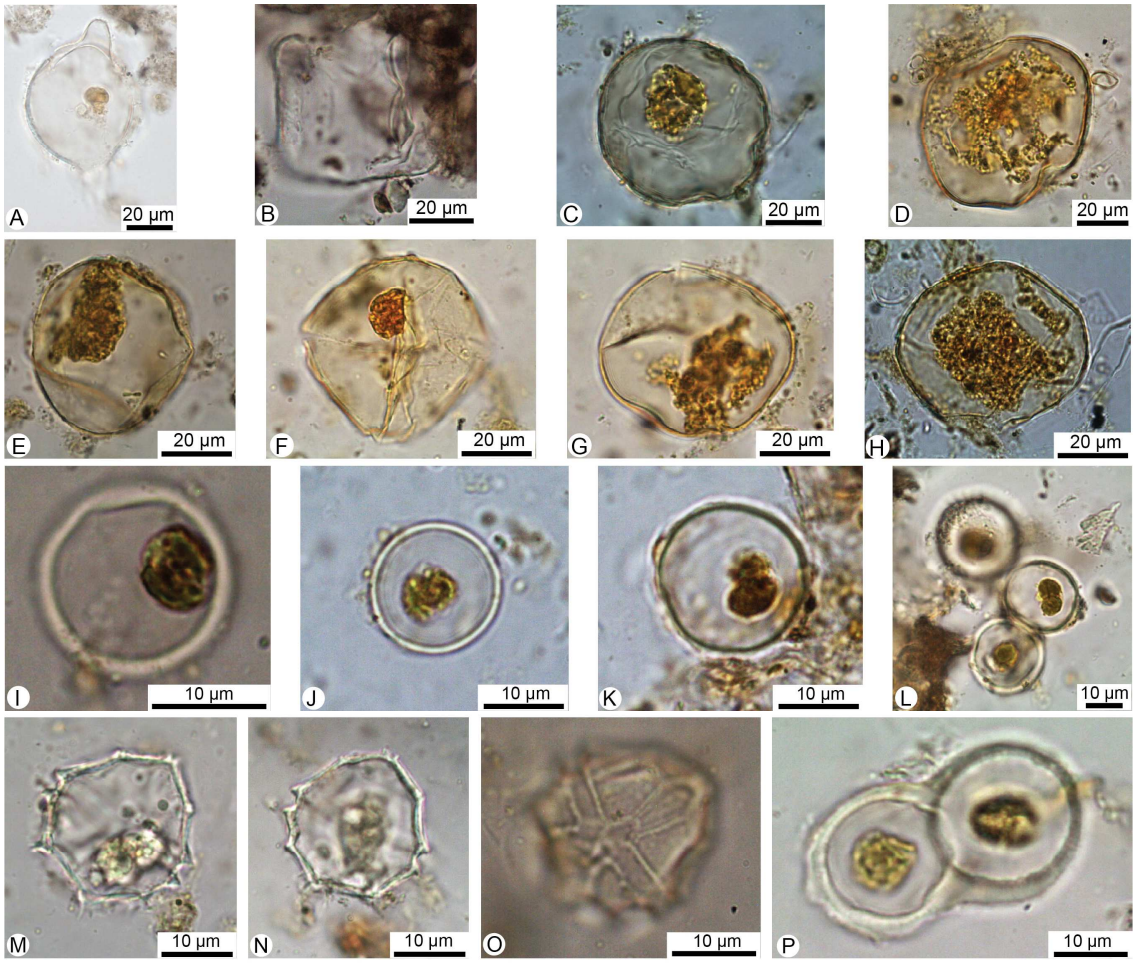

Plate 1.

Plate 4.1: Dinocyst and thecae taxa in Crawford Lake

A,B Cavate, proximate cysts of *Peridinium wisconsinense*, easily identified from their rounded, sometimes bifurcated apical horn and a single, sharply pointed antapical horn, measuring ~62 µm from horn to horn.
C,D Cavate, proximate cysts of *Peridinium willei*, with the outer layer slightly invaginated in the sulcal area to form two distinct shoulders that together with the larger size (~50 by 60 µm) distinguish it from cysts of *P. volzii.*
E-H *Peridinium volzii* small (~30 by 40 µm), unornamented cavate, proximate cysts with distinctive red bodies (F).
I-L Tiny (~16-20 µm in diameter) unornamented spherical cysts of *Parvodinium inconspicuum*, commonly containing red bodies.
M-N Ventral and dorsal views of a small (~20-25 µm by 20-30 µm) theca of *Parvodinium inconspicuum* (Lemmermann) Carty, showing the pronounced apical horn and scattered antapical spines. These are preserved in palynological preparations of varved sediments from Crawford Lake.
O Ventral perspective of a *Parvodinium inconspicuum* theca with clearly identifiabletabulation.
P Hypnozygotes of *Parvodinium inconspicuum* just completing a meiotic division with spongy walls and vivid orange body centrally located.

Plate 4.2: Pollen taxa in Crawford Lake indicative of land disturbance and agriculture

A *Helianthus* sp.- Sunflower Crawford Lake freeze core, 64 cm, a staple crop in Iroquios agriculture.
B *Ambrosia* sp.- Ragweed Crawford Lake freeze core, 19 cm, indicative of Euro-Canadian settlements due to their mass clear cutting of forests and tilling of fields.

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5. GENERAL CONCLUSIONS: THE POTENTIAL OF DINOCYSTS AS PROXIES OF EUTROPHICATION IN LACUSTRINE ENVIRONMENTS

The management of lakes requires an understanding of the limnological trends and their response to natural environmental changes and to past management interventions. Time series of environmental data in the microfossil record allow the response of critical freshwater resources to environmental perturbations (both natural and anthropogenic) to be assessed in a way that synoptic measurements of water quality cannot. Marine dinoflagellate cysts (dinocysts) have been used as proxies of cultural eutrophication in coastal environments, while the cysts of freshwater dinoflagellates have received little attention by the scientific community compared to their marine counterparts and to other paleolimnological proxies.

Because freshwater dinoflagellates are common phytoplankton in lacustrine ecosystems and their resting cysts have been reported in sediments of Oligocene to recent age, they have the potential to be a very useful and efficient tool in paleolimnology. However issues impede their exploitation as paleoenvironmental proxies, including: 1) the identification of both thecae and cysts is not straightforward due to infraspecific variation; 2) theca-cyst relationships remain poorly understood, largely isolating the phycologists and palynologists working on freshwater dinoflagellates; and 3) it is not known how many species of freshwater dinoflagellates produce fossilizable resting cysts, and the taphonomic factors controlling their distribution in sediments are poorly understood.

Recent studies in the Great Lakes region illustrate the potential of dinoflagellates as paleolimnological proxies using a combined phycological and pallynological approach, relating the stratigraphic and biogeographic distribution of cysts of several species of *Peridinium* to environmental conditions in lakes with documented anthropogenic impact. Cultural eutrophication associated with both Euro-Canadian colonists (since the mid 19th century) and previously by aboriginal inhabitants. Crawford Lake, Ontario, provides an ideal natural laboratory to study the response of freshwater dinoflagellates to cultural eutrophication. This unique body of water is located approximately 1 km west of the edge of the Niagara Escarpment in a conservation park and Iroquoian village learning center run by Conservation Halton. The anoxic bottom waters that result from meromixis in this small (2.4 ha) but deep (24 m) lake preserve varved sediments that host an exceptional fossil record. These annual layers provide dates for human activity (agriculture and land disturbance) around the lake over the last

millennium by both Iroquoian village farmers (ca. A.D. 1268-1486) and Canadian farmers beginning ~A.D. 1883. The excellent chronology and well established separate intervals of human activity around Crawford Lake, together with an abundance of available data from other fossil groups, allow us to further investigate the potential use of the cyst of freshwater dinoflagellates in studies of eutrophication.

The sediment record of Crawford Lake is exceptional, facilitating the preservation of dinoflagellate thecae and cysts. Dinocyst abundance covaries with abundance of diatoms and rotifer loricas and thus they are indicators of eutrophication. Eutrophication began in the 13th century when Iroquois settlements in and around the Crawford Lake catchment area impacted the lake ecosystem. Since then the eutrophic status of the lake has persisted but the relative paucity of dinocysts AD 1500-1850 reflects a reduced nutrient input. In the late 19th and 20th centuries, there was enhanced eutrophication of the lake.

Dinoflagellate cysts are found in slides processed for pollen analysis, which remains the best proxy of human impact in the watershed, so their identification by palynologists would prove useful in reconstructing pre- and post- impact conditions in the water bodies where the pollen accumulates. Dinocysts could be valuable in the management of lakes, since the response of critical freshwater resources to environmental perturbations (both natural and anthropogenic) can be assessed using time series of environmental data recorded by microfossils preserved in lake sediments in a way that synoptic measurements of water quality cannot. They thus appear to have the potential to be a useful and efficient tool in studies of eutrophication, as they have in marine environments. When combined into a multi-proxy they become a very viable and reliable identification tool for anthropogenic caused eutrophication

Further research at Crawford Lake should include collecting surface and water samples to determine which species of dinoflagellates that live within the water column. Comparison of the phycological data with the palynological record of cysts (and thecae) in the surface sediments would not only assist in confiiig theca-cyst relationschips, but would provide much-needed information regarding the number of species of freshwater dinoflagellates that produce fossilizable cysts. Cyst-theca relationships should also be confirmed by germinating cysts found in the surface sediments at Crawford Lake in a controlled laboratory setting, and their identification should also be confirmed using single cell-LSU rDNA analysis. Samples should be cultured downcore to strengthen the results obtained by inadvertent culturing (Chapter 3). Cysts in sediments as old as ~A.D.

1845-1860 (varve age) excysted, but systematic culturing should allow us to determine the longevity of cysts, at least in the exceptional setting of Crawford Lake, which may extend the viability beyond >150 years. Culturing should also be conducted at different times of year to see if an internal endogenous clock triggers excystment.

To further validate dinocysts as viable paleolimnological toosl for cultural eutrophiocation it would be beneficial to due downcore studies at various lakes throughout Ontario thus building on our knowledge of their biogeographical distribution as well as the water regimes each species thrives in.

Appendix 1.a Raw dinoflagellate cyst and theca counts, Crawford Lake core (Jan. 25, 2011).

Spike (dino)	100	100	100	100	100	100	100	100	100	100	100
Depth (cm)	**1**	**4**	**9**	**14**	**17**	**21**	**25**	***27***	**30**	***32***	**37**
AD	2010	2001	1980	1950	1935	1915	1891	1875	1867	1820	1610
Pwillei	2	3	1	3	2	2	2	4	11	5	5
P wisc	1	1		1			1	1	1	2	5
P. volzii	92	116	43	67	38	17	19	7	6	2	
P. inconspicuum	47	16	16	118	35	201	829	608	314	99	76
P. inconspicuum theca	1				2	34	25		1		
Total	143	136	60	189	77	254	876	620	333	108	86
cysts/ml	6206	5902	2604	8202	3341	11023	38018	26908	14452	4687	3732

spike (dino)	151	200	198	170	100	100	100	100	138	100
Depth (cm)	**42**	**45**	**52**	**57**	**59**	**62**	**64**	**67**	**69**	**72**
AD	1520	1500	1486	1410	1350	1305	1290	1220	1130	1040
Pwillei	3	7	1	7	3	2	2	6	1	4
P wisc	3	3	2	5	1			3		9
P. volzii										
P. inconspicuum	55	92	57	104	562	265	1064	89	62	189
P. inconspicuum theca								9		
Total	61	102	60	116	566	267	1066	107	63	202
cysts/ml	2647	4427	2604	5034	24564	11587	46264	4643	2734	8766

Appendix 1.b Calculated relative abundance (percentage) of cysts, Crawford Lake core (Jan. 25, 2011). Relative abundances are based on diniocyst sums of at least 60 cysts.

Depth (cm)	**1**	**4**	**9**	**14**	**17**	**21**	**25**	***27***	**30**	***32***	**37**
Total	142	136	60	189	75	220	851	620	332	108	86
P. willei	1	2	2	2	3	1	0.2	0.6	3	4	6
P. wisconsinense	0.7	0.7	0	0.5	0	0	0.1	0.1	0.3	2	6
P. volzii	65	85	72	35	51	8	2	1	1	2	0
P. inconspicuum	33	12	27	62	47		97	98	94	92	88
theca	0.7	0	0	0	3	15	3	0	0.3	0	0
cysts/ml	6163	5902	2604	8203	3255	9548	36933	26908	14408	4687	3732
spike (dino)	100	100	100	100	100	*100*	100	100	170	100	100

Depth (cm)	***42***	**45**	**52**	**57**	**59**	**62**	**64**	**67**	**69**	**72**
Total	*61*	102	60	116	566	267	1066	107	63	202
P. willei	5	7	1.7	6	0.5	0.7	0.1	6	2	2
P. wisconsinense	5	3	3.3	4	0.1	0	0	3	0	4.
P. volzii	*0*	0	0	0	0	0	0	0	0	0
P. inconspicuum	90	90	95	85	90	99	100	83	98	94
theca	*0*	0	0	0	0	0	0		0	0
cysts/ml	2647	4427	2604	5034	24564	11588	46264	4644	2734	8767
spike (dino)	151	200	198	170	100	100	100	100	138	100

Appendix 1.c Calculated concentrations (absolute abundance) of cysts and thecae, Crawford Lake core (Jan. 25, 2011).

Spike (dino)	100	100	100	100	100	100	100	100	170	100	100
Depth (cm)	**1**	**4**	**9**	**14**	**17**	**21**	**25**	***27***	**30**	**32**	**37**
cysts/ml	6162	5902	2604	8202	3341	9548	38018	*26908*	14452	4687	3732
P. willei	86	1302	43	130	86	86	86	*173*	477	217	217
P. wisconsinense	434	43	0	43	0	0	43	*43*	43	86	217
P. volzii	3992	5034	1866	2907	1649	737	824	*303*	260	86	0
P. inconspicuum	2039	694	694	5121	1519	8723	35979	*26387*	13628	42966	3298
P. inconspicuum theca	43	0	0	0	86	1475	1085	*0*	43	0	0

spike (dino)	151	200	198	170	100	*100*	100	100	138	100
Depth (cm)	**42**	**45**	**52**	**57**	**59**	***62***	**64**	**67**	**69**	**72**
cysts/ml	2647	4427	2604	5034	24564	*11587*	46264	4643	2734	8766
P. willei	130	304	43	304	130	*86*	86	260	43	173
P. wisconsinense	130	130	87	217	43	*0*	0	130	0	390
P. volzii	0	0	0	0	0	*0*	0	0	0	0
P. inconspicuum	2387	3993	2474	4514	24390	*11501*	46177	3862	2691	8202
P. inconspicuum theca	0	0	0	0	0	0	0	390	0	0

Appendix 2a. Raw pollen and spore counts, Crawford Lake core (Jan. 25, 2011). An initial quick count was done by Dr. Francine McCarthy to determine times of human habitation based on indicator herb pollen. Further pollen counts for statistical viability were done by myself.

Crawford Lake							
AD	2001	1915	1875	1867	1820	1610	1520
Depth (cm)	**4**	**21**	**27**	**30**	**32**	**37**	**42**
Cupress		2			11		
Pinus	29.5	10	73	112.5	91	62	46
Picea	2					3	8
Abies	1						
Tsuga	6	3	14	19	14	17	23
Betula	59	37	21	37	28	68	31
Ostrya	15	6	6	20	4	10	6
Corylus		1					
Carya	10	2	4	5	6	5	2
Tilia	2	1	13	6	5	5	9
Ulmus	24	21	23	19	8	18	13
Alnus	9	11	5	10	3	11	4
Juglans	6		9	1		1	
Quercus	25	21	26	37	20	34	42
Acer	24	15	17	29	17	1	19
Salix	1			2			
Fraxinus	7	6	7	11	7	8	9
Fagus	6		17	5	12	5	9
Nyssa	11		4	2	4	4	2
Castanea							
Rumex	1		7	3			
Ambrosia	33	94	37	13			
Artemisia	7	1	7	1			3
Chenopod	6	2	7	1			
Gramineae	7	9	10	8			
Cyperaceae	3	4		2		1	
Typha		2	1				
Potamogeton							
Compositae (other)	6	5	1				
Heilanthus							
Portulaca	1						
spores				2			
TOTAL	301.5	253	304	345.5	230	253	226
pollen spike	81	106	97	43	31	34	50
total herb (NAP	63	117	63	25	0	1	3
total arboreal	238.5	136	240	318.5	230	252	223

Crawford Lake							
AD	1500	1410	1350	1305	1290	1220	1040
Depth (cm)	45	57	59	62	64	67	72
Cupress				2			2
Pinus	36	23	18	13	13	6	12
Picea	1.5			1	1	2	
Abies							
Tsuga	18	9	14	9	16	17	6
Betula	33	61	36	61	49	48	49
Ostrya	6	5	4	2	2	5	4
Corylus	1			1			1
Carya	9	9	11	12	1	16	8
Tilia	8	4	8	4	9	20	13
Ulmus	13	6	12	8	16	22	19
Alnus	7	4	2	4	6	7	9
Juglans	2	1	5	1	3	1	3
Quercus	50	23	39	20	30	34	24
Acer	15	43	33	17	26	29	22
Salix		1	2	1	1	4	2
Fraxinus	9	4	12	5	12	6	17
Fagus	7	18	21	17	30	37	46
Nyssa		13	1	20	6	6	10
Castanea				1			
Rumex		5	1			2	
Ambrosia	1	1		2	5	1	
Artemisia		6	1	1	3	3	1
Chenopod		2		2	1	3	
Gramineae	1	3	5	2	2		4
Cyperaceae		1				1	
Typha							
Potamogeton		1					1
Compositae (other)		5	2	1			
Heilanthus	1	8	4	1	2		
Portulaca		1	1			1	
spores	1	1					
TOTAL	219.5	258	232	208	234	271	253
pollen spike	20	73	38	84	63	32	37
total herb (NAP	3	33	14	9	13	11	6
total arboreal	215.5	224	218	199	221	260	247

Appendix 2.b Calculated relative abundance (percentage) of pollen, Crawford Lake core (Jan. 25, 2011). Relative abundances are based on pollen and spore sums of at least 60.

Crawford Lake AD	2001	1915	1875	1867	1820	1610	1520
Depth (cm)	4	21	27	30	32	37	42
TOTAL	301.5	253	304	345.5	230	253	226
pollen spike	81	106	97	43	31	34	50
total herb (NAP	63	117	63	25	0	1	3
total arboreal	238.5	136	240	318.5	230	252	223
Cupress	0	1.5	0	0	4.7	0	0
Pinus	12.4	7.4	30.4	35.3	39.5	24.6	20.6
Picea	0.8	0	0	0	0	1.2	3.6
Abies	0.41	0	0	0	0		
Tsuga	2.5	2.2	5.8	6	6.0		
Betula	24.7	27.2	8.8	11.6	12.1	27	13.9
Ostrya	6. 3	4.4	2.5	6.3	1.7	4	2.7
Corylus	0	0.7	0	0	0	0	0
Carya	4. 2	1.5	1.7	1.6	2.6	2	0.9
Tilia	0.8	0.7	5.4	1.9	2.1	2	4.0
Ulmus	10. 1	15.4	9.5	6	3.4	7.1	5.8
Alnus	3. 8	8.1	2.	3.1	1.3	4.4	1.8
Juglans	2.5	0	3.8	0.3	0	0.4	0
Quercus	10.5	15.4	10.8	11.6	8.6	13.5	18.8
Acer	10. 1	11.0	7.1	9.1	7.3	0.4	8.5
Salix	0.4	0	0	0.6	0	0	0
Fraxinus	2.9	4.4	2.9	3.5	3.0	3.2	4.0
Fagus	2.5	0	7.1	1.6	5.2	2	4.0
Castanea	4.6	0	1.7	0.6	1.7	1.6	0.9
Cornus	0	0	0	0	0	0	0
Rumex	0.4	0	0.4	0.9	0	0	0
Ambrosia	13.8	69.1	15.4	4.1	0	0	0
Artemisia	2.99	0.7	2.9	0.3	0	0	1.3
Chenopod	2.5	1.5	2.9	0.3	0	0	0
Gramineae	2.9	6.6	4.2	2.5	0	0	0
Cyperaceae	1.2	2.9	0	0.6	0	0.4	0
Typha	0	1.5	0.4	0	0	0	0
Plantago	0	0	0	0	0	0	0
other aquatic	2.5	3.7	0.4	0	0	0	0
trilete spore	0	0	0	0	0	0	0
Dryopteris-type	0.4	0	0	0	0	0	0

Crawford Lake AD	1500	1410	1350	1305	1290	1220	1040
Depth (cm)	45	57	59	62	64	67	72
TOTAL	219.5	258	232	208	234	271	253
pollen spike	20	73	38	84	63	32	37
total herb (NAP	3	33	14	9	13	11	6
total arboreal	215.5	224	218	199	221	260	247
Cupress	0	0	0	1.0	0	0	0.8
Pinus	16.7	10.3	8.3	6.5	5.9	2.3	4.9
Picea	0.7	0	0	0.5	0.5	0.8	0
Abies	0	0	0	0	0	0	0
Tsuga	8.4	4.0	6.4	4.5	7.2	6.5	
Betula	15.3	27.2	16.5	30.7	22.2	18. 5	19.8
Ostrya	2.8	2.2	1.8	1.0	0.9	2	1.6
Corylus	0.5	0	0	0.5	0	0	0.4
Carya	4.2	4.0	5.0	6.0	0.5	6.2	3.2
Tilia	3.7	1.8	3.7	2.0	4.1	7.7	5.3
Ulmus	6.0	2.7	5.5	4.0	7.2	8.5	7.7
Alnus	3.2	1.8	0.9	2.0	2.7	2.7	3.6
Juglans	0.9	0.4	2.3	0.5	1.4	4	
Quercus	23.2	10.3	17.9	10.1	13.6	13. 1	9.7
Acer	7	19.2	15.1	8.5	11.8	11.2	8.9
Salix	0	0.4	0.9	0.5	0.5	1.5	0.8
Fraxinus	4.2	1.8	5.5	2.5	5.4	2.3	6.9
Fagus	3.2	8.0	9.6	8.5	13.6	14.2	18.6
Castanea	0	5.8	0.5	10.1	2.7	2.3	4.0
Cornus	0	0	0	0.5	0	0	0
Rumex	0	2.2	0.5	0	0	0.8	0
Ambrosia	0.5	0.4	0	1.0	2.3	4	
Artemisia	0	2.7	0.5	0.5	1.4	1.6	0.4
Chenopod	0	0.9	0	1.0	0.5	1.6	0
Gramineae	0.5	1.3	2.3	1.0	0.9	0	1.6
Cyperaceae	0	0.4	0	0	0	0.4	0
Typha	0	0	0	0	0	0	0
Plantago	0	0.4	0	0	0	0	0.4
other aquatic	0	2.2	0.9	0.5	0	0	0
trilete spore	0.5	3.6	1.8	0.5	0.9	0	0
Dryopteris-type	0	0.4	0.5	0	0	0.4	0

Appendix 2.c Calculated concentrations (absolute abundance) of pollen, Crawford Lake core (Jan. 25, 2011).

Depth (cm)	4	21	27	30	32	37	42
TOTAL pollen	301.5	253	304	345.5	230	253	226
herbs	22	78	25	7	0	0	1
AD	2001	1915	1875	1867	1820	1610	1520
pollen/ml	12779	5568	10738	32146	32200	32167	19356
Cupress	0	82	0	0	1540	0	0
Pinus	1581	409	3266	11355	12740	7914	3993
Picea	107	0	0	0	0	383	694
Abies	54	0	0	0	0	0	0
Tsuga	321	123	626	1918	1960	2170	1996
Betula	3161	1515	939	3734	3920	8680	2691
Ostrya	804	246	268	2019	560	1276	521
Corylus	0	41	0	0	0	0	0
Carya	536	82	178	505	840	638	174
Tilia	107	41	581	606	700	638	781
Ulmus	1286	860	1029	1918	1120	2298	1128
Alnus	482	450	224	1009	420	1404	347
Juglans	321	0	403	101	0	128	0
Quercus	1340	860	1163	3734	2800	4340	3646
Acer	1286	614	761	2927	2380	128	1649
Salix	54	0	0	202	0	0	0
Fraxinus	375	246	313	1110	980	1021	781
Fagus	321	0	761	505	1680	638	781
Castanea	589	0	179	202	560	511	174
Cornus	0	0	0	0	0	0	0
Rumex	54	0	45	303	0	0	0
Ambrosia	1768	3849	1655	1312	0	0	0
Artemisia	375	41	313	101	0	0	261
Chenopod	321	82	313	101	0	0	0
Gramineae	375	368	447	807	0	0	0
Cyperaceae	161	164	0	202	0	128	0
Typha	0	82	45	0	0	0	0
Plantago	0	0	0	0	0	0	0
other aquatic	321	205	45	0	0	0	0
trilete spore	0	0	0	0	0	0	0
Dryopteris-type	54	0	0	0	0	0	0
populus	511	1169	2899	9644	10304	11902	8130

Depth (cm)	45	57	59	62	64	67	72
TOTAL pollen	219.5	258	232	208	234	271	253
herbs	1	5	3	4		3	2
AD	1500	1410	1350	1305	1290	1220	1040
pollen/ml	46764	13317	24898	10282	15224	35263	28972
Cupress	0	0	0	103	0	0	235
Pinus	7812	1367	2056	672	896	814	1408
Picea	326	0	0	52	69	272	0
Abies	0	0	0	0	0	0	0
Tsuga	3906	535	1599	465	1102	2306	704
Betula	7161	3627	4112	3152	3376	6510	5748
Ostrya	1302	297	457	103	138	678	469
Corylus	217	0	0	52	0	0	117
Carya	1953	535	1256	620	69	2170	938
Tilia	1736	238	914	207	620	2713	1525
Ulmus	2821	357	1371	413	1102	2984	2229
Alnus	1519	238	228	207	413	949	1056
Juglans	434	59	571	52	207	136	352
Quercus	10850	1367	4454	1033	2067	4611	2815
Acer	3255	2556	3769	878	1791	3934	2581
Salix	0	59	228	52	69	543	235
Fraxinus	1953	238	1371	258	827	814	1994
Fagus	1519	1070	2398	878	2067	5018	5396
Castanea	0	773	114	1033	413	813.75	1173
Cornus	0	0	0	52	0	0	0
Rumex	0	297	114	0	0	271	0
Ambrosia	217	59	0	103	344	135.625	0
Artemisia	0	357	114	52	207	406.875	117
Chenopod	0	119	0	103	69	406.875	0
Gramineae	217	178	571	103	138	0	469
Cyperaceae	0	59	0	0	0	136	0
Typha	0	0	0	0	0	0	0
Plantago	0	59	0	0	0	0	117
other aquatic	0	297	228	52	0	0	0
trilete spore	217	476	457	52	138	0	0
Dryopteris-type	0	59	114	0	0	136	0
populus	21044		14690	6375	9744	23626	